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# DO NEMATODES ASSIST BACTERIAL INVASION OF THE HOST BY WOUNDING THE WALL OF THE INTESTINAL TRACT?

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In a paper by Taylor and Purchase (1931) a short account was given of the previous works on the importance of parasitic worms in producing infection with bacterial diseases. It might be of some value to cite what has been more recently published before proceeding to record the experiments which have just been completed.

In 1929 Joyeux and Baer carried out some experiments on the transference of the plerocercoid of *Diphyllobothrium ranarum* from one animal to another, and were surprised to observe that, in spite of the grave trauma which must have been caused by this relatively large larval stage of a cestode passing through the wall of the stomach or intestine, bacterial invasion of the peritoneum did not result. They have shown, however, that the sparganum produces some bactericidal substance capable of inhibiting the growth of bacteria in broth.

On making a post-mortem examination of an ox, Kinsley (1931) found oesophagostomes in the bowel, and was also able to recover bipolar-staining organisms from the heart blood. He concluded that internal parasitism was the primary cause of the trouble, and that haemorrhagic septicaemia was the cause of death.

In a paper by Le Roux (1932) appears the following: "Recently I observed cases of Quarter Evil in cattle which had evidently become infected with the organisms of that disease through the agency of the Liver Fluke found in the grass."

Skrjabine (1932) cites "the opening of the door to infections and contributing to bacterial diseases" as one of the four principal ways in which parasitic worms do harm.

Shortt, Campbell and Lal (1932) failed to infect twenty-seven Chinese hamsters with kala-azar by administering large numbers of hookworm larvae cultivated in the presence of Leishman-Donovan bodies.

Carne and Ross (1932) have administered the infective larvae of *Oesophagostomum columbianum* to lambs, *per os*, along with cultures of the Priesz-Nocard bacillus, but in spite of marked lesions of oesophagostomiasis of the bowel wall and the presence of considerable numbers of other intestinal nematodes, they could find no evidence of the parasitic worms playing any part in bringing about infection with the Priesz-Nocard bacillus.

Bassett and Moulin (1933) found *Bacillus coli* and *B. viscosum* in the blood, in the liver and in the bowel wall of young mules which had died of some acute disease. The bacteria in the bowel wall were found in the proximity of lesions caused by strongyloid nematodes, and the authors concluded that the worms were probably responsible for their introduction.

Koidzumi (1927) reports that Ugami and Miyagawa carried out a series of experiments on guinea-pigs. These were first moderately infected with *Ascaris* larvae, and then, 17 days later, given an intraperitoneal injection of tubercle bacilli. The results which they obtained were contrary to what had been expected in that the controls became more severely infected with tuberculosis than did those which were infected with *Ascaris*.

It is thus seen that, on the one hand, four more reports of observations showing parasitic worms to have no action in introducing bacteria, and, on the other hand, four more expressions of opinion that they play an important rôle in this respect are added to the several which were previously cited by Taylor and Purchase (1931). These authors carried out experiments to ascertain whether nematode larvae, in penetrating the intestinal mucosa, played any part in assisting pathogenic bacteria to invade the host from the lumen of the bowel. The results which they obtained were, however, completely negative, although the conditions of their experiment were designed in such a way as to provide for the passage of pathogenic bacteria along the intestinal tract during the time that large numbers of nematode larvae were actively penetrating the mucosa. The reason suggested for this unexpected result was that the injury caused by the passage of the minute larvae through the tissues was not enough to permit the ingress of sufficient bacteria to bring about infection. It was therefore thought worth while repeating the experiments with larger parasites which would produce more extensive wounds in the tissues; and the following is an account of the work which has been carried out.

*Bacillus suipestifer* in the rabbit appears to be a suitable combination of infective organism and susceptible host for the purposes of this experiment, as a fatal infection can easily be produced by the administration of a sufficient dose of a broth culture by the mouth, although a lower dose produces no such effect. It is reasonable to suppose, therefore, that the injuries to the mucous membrane caused by adult parasitic worms might give access to the bacteria and by so doing bring about infection through what would otherwise be a sub-infective dose.

Two separate species of parasitic worm were employed, one *Graphidium strigosum*, which occurs in the stomach, and the other *Trichostrongylus retortaeformis*, which occurs in the small intestine.

### Exp. 1. Using *Graphidium strigosum*

This trichostrongylid nematode, which is common in rabbits and hares in Europe, is a comparatively stout worm, about 1 in. in length, of a bright red colour, and having a mouth which is comparatively large for members of this

sub-family. On removing these worms from the wall of the stomach, bright red spots may be seen at the points where they have been attached; if they are macerated in water, haemoglobin can easily be demonstrated and it is clear that they withdraw blood. Sections of the stomach wall show very extensive damage to the mucous membrane and the penetration of some of the worms into the muscular coat. It is, however, remarkable that they should be able to cause such extensive injury without calling forth any signs of an inflammatory reaction.

Table I. *Giving protocols of the experiment with* Graphidium strigosum *and* Bacillus suipestifer *in rabbits.*

*Bacillus suipestifer* was recovered in pure culture from the heart blood of all of the rabbits which died, but there is no evidence of the parasitic worms having played any part in introducing the bacteria, the rate of mortality being approximately equal in both groups.

| No. of rabbit | Relative dose of suspension of infective larvae in c.c. | Date, Nov. | | No. of *Graphidium* recovered at autopsy | Relative infestation of *Passalurus* found at autopsy |
|---|---|---|---|---|---|
| | | Died | Killed | | |
| 1 | 1 | 6 | — | 172 | Very light |
| 2 | 1 | — | 28 | 213 | Very heavy |
| 3 | 2 | 6 | — | 302 | Heavy |
| 4 | 2 | 7 | — | 346 | Heavy |
| 5 | 3 | 16 | — | 573 | Heavy |
| 6 | 3 | — | 28 | 463 | Very heavy |
| 7 | 4 | — | 28 | 572 | Very light |
| 8 | 4 | 7 | — | 367 | Heavy |
| 9 | 5 | 10 | — | 850 | Light |
| 10 | 5 | 6 | — | 640 | Heavy |
| 11 | 6 | 7 | — | 893 | None |
| 12 | 6 | 8 | — | 294 | Heavy |
| 13 | — | — | 28 | — | None |
| 14 | — | — | 28 | — | Light |
| 15 | — | 5 | — | — | None |
| 16 | — | — | 28 | — | Very light |
| 17 | — | 5 | — | — | Light |
| 18 | — | 7 | — | — | Heavy |
| 19 | — | — | 28 | — | Heavy |
| 20 | — | 6 | — | — | Heavy |
| 21 | — | 6 | — | — | Heavy |
| 22 | — | 6 | — | — | None |
| 23 | — | 12 | — | — | Heavy |
| 24 | — | 10 | — | — | Very light |

The doses of infective larvae were given on the following dates: June 30, July 9, 16, 28, August 8, 13, 22, 29, and September 15.

Each rabbit received a dose of 1/100 c.c. of a 24 hours' broth culture of *Bacillus suipestifer*, by mouth, on September 30.

Twenty-four rabbits 8–10 weeks old were used in this experiment. They were divided into two equal groups, one only of which received *Graphidium* larvae. These were given in this group on nine occasions spread over a period of 11 weeks, the doses being graded on each occasion, as shown in Table I, in order to provide for the eventuality of a heavy infestation being more effective than a light one. Fifteen days after the last dose of infective larvae had been administered, a fast of 12 hours was enforced, after which all rabbits, in both the test group and the control group, received, through the stomach tube, a dose of 1/100 c.c. of a 24 hours' broth culture of virulent

*Bacillus suipestifer*, suspended in 2 c.c. of normal saline. The results of this experiment are detailed in Table I, and it will be seen that they were completely negative.

### Exp. 2. Using *Trichostrongylus retortaeformis*

This parasitic worm is much smaller than *Graphidium* and infests the first portion of the small intestine. In large numbers it is definitely pathogenic and may lead to the death of the rabbit.

*Trichostrongylus retortaeformis* like *Graphidium strigosum* withdraws blood from the mucosa, and haemoglobin can easily be demonstrated in washings of the pounded worms; although it does not cause obvious lesions, nor penetrate the mucous membrane, its presence appears to be irritating to the tissues as a heavy infestation frequently causes enteritis.

Fifty-one rabbits 8–10 weeks old were divided as evenly as possible as regards size and age into two groups, a test group of twenty-seven, and a control group of twenty-four. The test group was subdivided into three equal groups of nine, which subsequently received doses of infective larvae of *Trichostrongylus retortaeformis* in the proportions of 1, 2 and 4 respectively as shown in Table II. Three doses were given at intervals of 3 days to the rabbits in the test group, and 18 days after the last dose all the rabbits, both in the test group and in the control group, received, by stomach tube, a dose of 1/300 c.c. of a 24 hours' broth culture of virulent *Bacillus suipestifer* suspended in 2 c.c. of normal saline—the dose having been lowered on account of the large number of deaths which had occurred in the previous experiment.

Details of the doses of infective larvae and of the results obtained are set out in Table II. They fail to indicate that the worms played any part in introducing infection.

### The length of time *Bacillus suipestifer* retains its virulence in the intestinal tract

For the proper interpretation of these results it is essential to know the length of time during which the pathogenic bacteria remained alive in the intestine of the rabbit, in contact with the parasitic worms.

It having been ascertained in a preliminary trial that the spores of lycopodium, which are easily recognisable under the microscope, can be recovered from the ingesta by the ordinary floatation technique, used for the concentration of nematode eggs, a suspension of this powder was mixed with the dose of bacteria given to the rabbit and so provided a ready means of ascertaining its distribution at the time of death. Six rabbits were dosed in this way, each receiving 3/4 c.c. of a 24 hours' broth culture of virulent *B. suipestifer* along with 1/4 c.c. of a suspension of lycopodium spores. The rabbits were killed 1/4, 1/2, 1, 2, 4 and 6 hours respectively, after receiving the dose; autopsy was immediately carried out and ingesta collected separately from stomach, small intestine and large intestine in each instance. A little physiological saline was

Table II. *Giving protocols of the experiment with* Trichostrongylus retortaeformis *and* Bacillus suipestifer *in rabbits.*

*Bacillus suipestifer* was recovered in pure culture from the heart blood of all of the rabbits which died.

Whereas eight of the nine rabbits carrying the most worms died, and seven of the nine carrying an intermediate number, only three of the nine carrying least worms died; as, however, seventeen of the twenty-four controls also died, it is apparent that the parasitic worms did not play any important part in introducing infection.

| No. of rabbit | No. of *Trichostrongylus* larvae given to the rabbits | | | Date of death, Mar. | No. of *Trichostrongylus* recovered at autopsy |
|---|---|---|---|---|---|
| | Feb. 21 | Feb. 24 | Feb. 27 | | |
| 1 | 527 | 930 | 240 | — | — |
| 2 | 527 | 930 | 240 | — | — |
| 3 | 527 | 930 | 240 | 25 | 1500 |
| 4 | 527 | 930 | 240 | — | — |
| 5 | 527 | 930 | 240 | — | — |
| 6 | 527 | 930 | 240 | 24 | 1200 |
| 7 | 527 | 930 | 240 | — | — |
| 8 | 527 | 930 | 240 | — | — |
| 9 | 527 | 930 | 240 | 25 | 1350 |
| 10 | 1054 | 1860 | 480 | — | — |
| 11 | 1054 | 1860 | 480 | 25 | 2900 |
| 12 | 1054 | 1860 | 480 | 26 | 1767 |
| 13 | 1054 | 1860 | 480 | 24 | 200 |
| 14 | 1054 | 1860 | 480 | 24 | 2950 |
| 15 | 1054 | 1860 | 480 | — | — |
| 16 | 1054 | 1860 | 480 | 23 | 2850 |
| 17 | 1054 | 1860 | 480 | 25 | 336 |
| 18 | 1054 | 1860 | 480 | 27 | 1850 |
| 19 | 2108 | 3720 | 960 | 27 | 5333 |
| 20 | 2108 | 3720 | 960 | 25 | 5550 |
| 21 | 2108 | 3720 | 960 | 24 | 5233 |
| 22 | 2108 | 3720 | 960 | — | — |
| 23 | 2108 | 3720 | 960 | 30 | 4900 |
| 24 | 2108 | 3720 | 960 | 26 | 3133 |
| 25 | 2108 | 3720 | 960 | 28 | 4333 |
| 26 | 2108 | 3720 | 960 | 23 | 2330 |
| 27 | 2108 | 3720 | 960 | 23 | 2467 |
| 28 | — | — | — | 27 | — |
| 29 | — | — | — | 26 | — |
| 30 | — | — | — | — | — |
| 31 | — | — | — | — | — |
| 32 | — | — | — | 24 | — |
| 33 | — | — | — | 25 | — |
| 34 | — | — | — | — | — |
| 35 | — | — | — | — | — |
| 36 | — | — | — | 25 | — |
| 37 | — | — | — | 24 | — |
| 38 | — | — | — | 27 | — |
| 39 | — | — | — | 23 | — |
| 40 | — | — | — | 23 | — |
| 41 | — | — | — | 25 | — |
| 42 | — | — | — | — | — |
| 43 | — | — | — | 25 | — |
| 44 | — | — | — | 24 | — |
| 45 | — | — | — | 27 | — |
| 46 | — | — | — | 26 | — |
| 47 | — | — | — | — | — |
| 48 | — | — | — | 25 | — |
| 49 | — | — | — | 25 | — |
| 50 | — | — | — | — | — |
| 51 | — | — | — | 25 | — |

Each rabbit received a dose of 1/300 c.c. of a 24 hours' broth culture of *Bacillus suipestifer* on March 17.

mixed with the collection of stomach and intestinal contents, and strained off through glass wool. 5 c.c. of the filtrate was then inoculated subcutaneously into a test rabbit and an ordinary helminthological centrifugal floatation test was carried out on the remainder, for the recovery of lycopodium spores, as evidence that the *B. suipestifer* had reached that part.

Table III. *Giving protocols of the experiment to ascertain the whereabouts of* Bacillus suipestifer *in the intestine at various times after its administration by mouth, and the length of time during which its infectivity was retained.*

The lycopodium spores, which were recovered by centrifugal floatation in strong salt solution, served to indicate the whereabouts of the bacteria, and the test rabbit to indicate their viability in various parts of the intestinal tract, and at various periods after the administration of the dose. Nine of the test rabbits are seen to have died, but in only two instances was *Bacillus suipestifer* recovered from the heart blood; this result is sufficient, however, to indicate that the bacilli were alive in the large intestine up to 6 hours after the administration of the dose.

| No. of rabbit | Interval between dose and autopsy | Relative no. of lycopodium spores in stomach, small and large intestine | No. of rabbit inoculated with 5 c.c. of filtrate of ingesta from stomach, small and large intestine | | Date, died or killed |
|---|---|---|---|---|---|
| 1 | Killed after ¼ hour | + + + | 7 | Stomach | — |
| | | + | 8 | Small intestine | — |
| | | + | 9 | Large intestine | — |
| 2 | Killed after ½ hour | + + + | 10 | Stomach | — |
| | | + | 11 | Small intestine | May 29 |
| | | + | 12 | Large intestine | — |
| 3 | Killed after 1 hour | + + + | 13 | Stomach | June 13 |
| | | + | 14 | Small intestine | — |
| | | + + | 15 | Large intestine | May 28 |
| 4 | Killed after 2 hours | + + + | 16 | Stomach | June 1 |
| | | + | 17 | Small intestine | — |
| | | + + | 18 | Large intestine | June 3 |
| 5 | Killed after 4 hours | + + + | 19 | Stomach | June 13 |
| | | + | 20 | Small intestine | — |
| | | + + + | 21 | Large intestine | June 23 |
| 6 | Killed after 6 hours | + + | 22 | Stomach | May 27 |
| | | + | 23 | Small intestine | — |
| | | + + | 24 | Large intestine | May 30 |

Rabbits Nos. 1 to 6 received 0·75 c.c. of a 24 hours' broth culture of *Bacillus suipestifer* and 0·25 c.c. of a suspension of lycopodium spores on May 25.

*Bacillus suipestifer* was recovered in pure culture from the heart blood of Nos. 18 and 24 but not from Nos. 11, 13, 15, 16, 19, 21 and 22.

Rabbits Nos. 13 and 19 were sick when killed.

The results of the experiment are set out in Table III. It will be seen that up to the end of the second hour most of the infective dose appeared to be in the stomach, at 4 and 6 hours it appeared to be about equally distributed between stomach and large intestine, and a certain amount was present in the small intestine throughout the whole of the 6 hours' period. The transference of the infective dose from the stomach to the large intestine therefore appears to have begun very quickly after its administration and to have continued for more than 6 hours.

The evidence obtained by these experiments shows that *B. suipestifer* was still alive in the contents of the large intestine up to 6 hours after the ad-

ministration of the dose, and it is evident that for some of that time, at least, the virulent bacteria must have been in the vicinity of the parasitic worms and probably in actual contact with that portion of the stomach and intestinal mucosa which was being attacked by the worms.

## SUMMARY AND CONCLUSIONS

The experiments here described make provision for some of the eventualities not covered by the previous experiments of Taylor and Purchase, and appear to give a more definite answer to the question on the effect of the presence of parasitic worms in aiding the entry of bacteria into the body.

Although the conditions, in these experiments, appear to have been very favourable for the bacteria present in the lumen of the bowel to invade the host through abrasions of the wall caused by the parasitic worms, the evidence of assistance given by the worms to the entry of bacteria has not been obtained. In view of these findings, and taking into consideration other experimental results, it seems highly probable that the injuries caused to the bowel wall by parasitic worms in general do not play any important part in bringing about bacterial infection from the lumen of the intestine.

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# THE TREMATODE PARASITES OF *TURRITELLA COMMUNIS* LMK. FROM PLYMOUTH AND NAPLES

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(With 22 Figures in the Text)

In 1931 Dr M. V. Lebour drew my attention to a larval Trematode (one of the "Hugetailed Monostome" group of H. M. Miller, 1925) which occurred quite frequently in *Turritella communis* from the Rame Mud, Plymouth. During 1932–4 I examined 541 *Turritella communis* from the same locality and an 8 per cent. general infection with four species of closely related cercariae was recorded, but the species which was common the previous year did not occur once. 180 *Turritella communis* from Naples revealed a 5 per cent. infection of two other closely related species.

Table I

| | No. examined | No. of ♂ | No. of ♀ | No. of ♂ infected | No. of ♀ infected | No. infected where sex undetermined | General infection % |
|---|---|---|---|---|---|---|---|
| *Turritella communis* from Plymouth | 541 | 216 | 324 | 37 | 7 | 1 | 8 |
| *Turritella communis* from Naples | 180 | 63 | 116 | 5 | 4 | 1 | 5 |

The *Turritella* measured from 15 to 46 mm. in length. The females were larger than the males and outnumbered them by 3 : 2. Despite this fact, and that only specimens over 30 mm. were infected, out of 53 infected individuals 42 were males (see Table I). These figures seem to indicate that the miracidia will most readily enter males. The seat of infection of these cercariae is in the gonads, and an explanation may be found in the fact that live sperms are present in the male gonads throughout the year whereas the ova only ripen periodically.

## Technique

Various techniques recommended by Dubois (1929), Stunkard (1930), Rees (1932), and Sewell (1922) were adopted. In addition cercariae were anaesthetised with menthol crystals. In this state spines, ducts of penetration glands, etc., were often seen to great advantage.

*Measurements.* These were taken in a relaxed state under a coverslip, at the moment when the pressure began to flatten out the fins. Only *Cercaria doricha* was successfully measured without a coverslip.

### MAIN CHARACTERS OF THE *RHODOMETOPA* GROUP

All six species of cercariae are closely related to each other and to *Cercaria rhodometopa* Pérez (1924), which was briefly recorded and named by Pérez from material obtained at Roscoff and in Morlaix Bay and redescribed by W. H. Stunkard (1932) from a single *Turritella* at Roscoff:

Cercariae large (over 500$\mu$ in body length) leaf-shaped, flattened dorso-ventrally, and swim actively. Cuticle spined, with additional cuticular tubercles. Oral and ventral suckers well developed. Pharynx, oesophagus and bifurcated intestine present, but often extremely difficult to detect. Pre-pharynx sometimes present. Excretory vesicle Y-shaped with lateral branched evaginations occupying almost entire body, and contains excretory granules. Excretory pore terminal. Flame-cell pattern:

$$2\,[(5+5+5+5+5+5)+(5+5+5+5+5+5)]=120$$

or $$2\,[(6+6+6+6+6+6)+(6+6+6+6+6+6)]=144.$$

Two or five groups of penetration glands present.

Reproductive organs poorly developed.

Tail about as long as body, with longitudinal fin membrane running along centre of dorsal and ventral surface, and about 50 lateral semicircular fins set at right angles to main longitudinal fins.

Pink pigment usually present.

Development in sausage-shaped, yellowish, immobile sporocysts, in gonad of host.

### SPECIFIC CHARACTERS OF THE CERCARIAE

(See Table II)

Distinguishing between larval forms of closely related Trematodes presents great difficulty. The best example of this is found in the cercariae of Notocotylidae, where in some cases simultaneous comparison of living specimens seems the only method of identification. My own belief, based on morphological and "behaviouristic" observations, is that there are seven species described in the *Rhodometopa* group (six in this paper). Only careful life-history studies can prove this satisfactorily. *Cercaria nicarete* may be a subspecies or a larval variety of *C. pythionike*, for although examples of the two have never been found in the same infection they might vary from snail to snail. Again, the considerably larger tails of both Mediterranean forms may be merely larval variations leaving no trace in the adult. Most of the specific differences, such as thickness of cuticle, degree of development of cuticular tubercles and spines, transparency of certain organs and opaqueness of others, degree of granulation of the penetration glands, and movements and activity under a coverslip, are really only appreciated when comparison of living material is possible or if the observer is familiar with the other species. One diagnostic character which has proved very useful is the maximum diameter of the excretory granules. These

Table II. *Identification of cercariae of* Rhodometopa *group*

| Species ... | *Cercaria pythionike* | *Cercaria doricha* | *Cercaria nicarete* | *Cercaria herpsyllis* | *Cercaria rhodometopa* | *Cercaria ampelis* | *Cercaria ranzii* |
|---|---|---|---|---|---|---|---|
| Pigment | Pink pigment present in anterior end | Generally none. If present only in tail | No pigment | Pink pigment as in *C. pythionike* | Pink pigment as in *C. pythionike* | Very faint specks of pink pigment in anterior end | Pink pigment as in *C. pythionike* |
| Situation of ventral sucker | Below middle of body length | Well above middle of body length | Below middle of body length | Below middle of body length | Below middle of body length | In the centre of body length | Slightly above middle of body length |
| Prepharynx | Absent | Small prepharynx sometimes developed | Absent | Absent | Well-developed prepharynx present | Absent | Absent |
| Pharynx, oesophagus and intestine | All fairly well developed | All fairly well developed | All fairly well developed | Intestine difficult to trace | Intestine absent | Pharynx and oesophagus poorly developed. Intestine very difficult to see | Pharynx and oesophagus poorly developed. Intestine very difficult to see |
| Lateral groups, penetration glands | Absent | Present | Absent | Absent | Absent | Absent | Absent |
| Excretory vesicle | Very few granules. Vesicle appears light on dark background | Packed with granules. Vesicle appears dark on light background | Very few granules. Vesicle appears light on dark background | Medium number of granules. Vesicle darkish on light background | ? | Many granules. Excretory vesicle dark on light background | Fewest granules. Excretory vesicle light on dark background |
| Diameter of maximum excretory granules | 9·5 $\mu$ | 2·9 $\mu$ | 11·1 $\mu$ | 3·4 $\mu$ | ? | 4 $\mu$ | 11 $\mu$ |
| Flame cells | 5 to each group | 6 to each group | 5 to each group | 5 to each group | 5 to each group | 5 to each group | 5 to each group |
| Lateral cuticular tubercles | Diminish below half of body length | Barely reach level of ventral sucker | Reach to posterior extremity of body | Diminish below half of body length | ? | Diminish below half of body length | Diminish below half of body length |

are remarkably constant in size in each species, and because of their "solid" nature can be measured easily and accurately. Their diameter, taken in conjunction with their number, is often invaluable, particularly as this character, unlike spines, cuticle, etc., can be considered before the emergence of the cercariae.

**Cercaria pythionike** n.sp. (Figs. 1, 3, 4, 5, 7, 13–20*a*)

Measurements in microns

| | |
|---|---|
| Body length ... ... ... ... ... ... | 1104–816 |
| Body width opposite ventral sucker ... ... ... | 440–400 |
| Tail length ... ... ... ... ... ... | 1120–880 |
| Tail width (maximum without fins) ... ... ... | 96– 95 |
| Lateral fins depth (maximum) ... ... ... ... | 64 |
| Dorsal fin width (maximum) ... ... ... ... | 144–143 |
| Ventral fin width (maximum) ... ... ... ... | 144–143 |
| Oral sucker ... ... ... ... ... ... | 88 × 96–96 × 98 |
| Ventral sucker ... ... ... ... ... ... | 80 × 80–82 × 82 |
| Pharynx ... ... ... ... ... ... ... | 32 × 40 |
| Oesophagus ... ... ... ... ... ... | 15 × 88 |
| Excretory granules (maximum) ... ... ... | 9·5 × 9·5 |
| Flame cell ... ... ... ... ... ... ... | 2·4 |
| Granules of sporocyst ... ... ... ... ... | 1·7 |

2 *per cent. infection.*

*General form and armature.* Body of cercaria (Figs. 1 and 7) when relaxed, oval in outline, tapering slightly anteriorly. Widest at base of ventral sucker. Capable of considerable expansion and contraction. Anterior end as far as termination of oesophagus bright rose red (Fig. 7). Pigmentation arises early in development, simultaneously with rudiments of suckers, attains maximum brilliance shortly before emergence.

Tail slightly longer than body, tapering to a point. Round in section; firmly inserted in slightly subterminal caudal pocket. Dorsal and ventral fin membranes attain maximum width about centre of tail and diminish towards extremity. Lateral fins somewhat irregular in size. Largest situated half-way along tail, smallest at either end. Giant cells with giant nuclei present along entire length of tail prior to emergence of cercaria. In fully developed specimens only remains of nuclei, in form of scattered granules, are present.

Body of cercaria covered with thick cuticle. Blunt semitransparent tubercles present in following positions:

Along lateral edges of body, each separated by average distance of 32$\mu$ except in region of oral sucker where they are more closely set. Below ventral sucker they diminish in size and are often absent.

Two parallel rows running from anterior end to about level of region of ventral sucker, below which they diminish and disappear.

Two pairs, one longer than the other, in region of oral sucker.

Asymmetrical group of sixteen exactly opposite ventral sucker.

Circle of nine on inner lip of both suckers.

Circle of twelve, outside, but roughly encircling oral sucker.

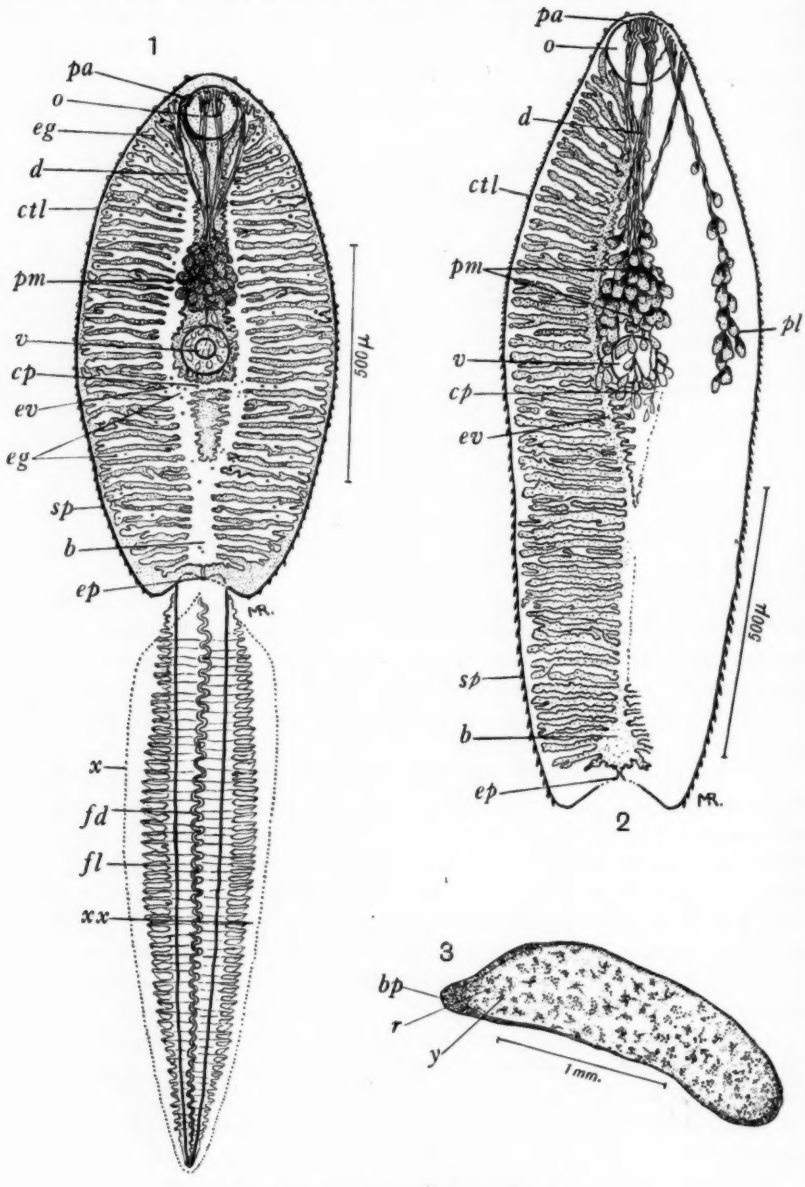


Fig. 1. *Cercaria pythionike:* general view (dorsal).
Fig. 2. *Cercaria doricha:* general view (dorsal), without tail.
Fig. 3. *Cercaria pythionike:* sporocyst.

For explanation of lettering see p. 169.

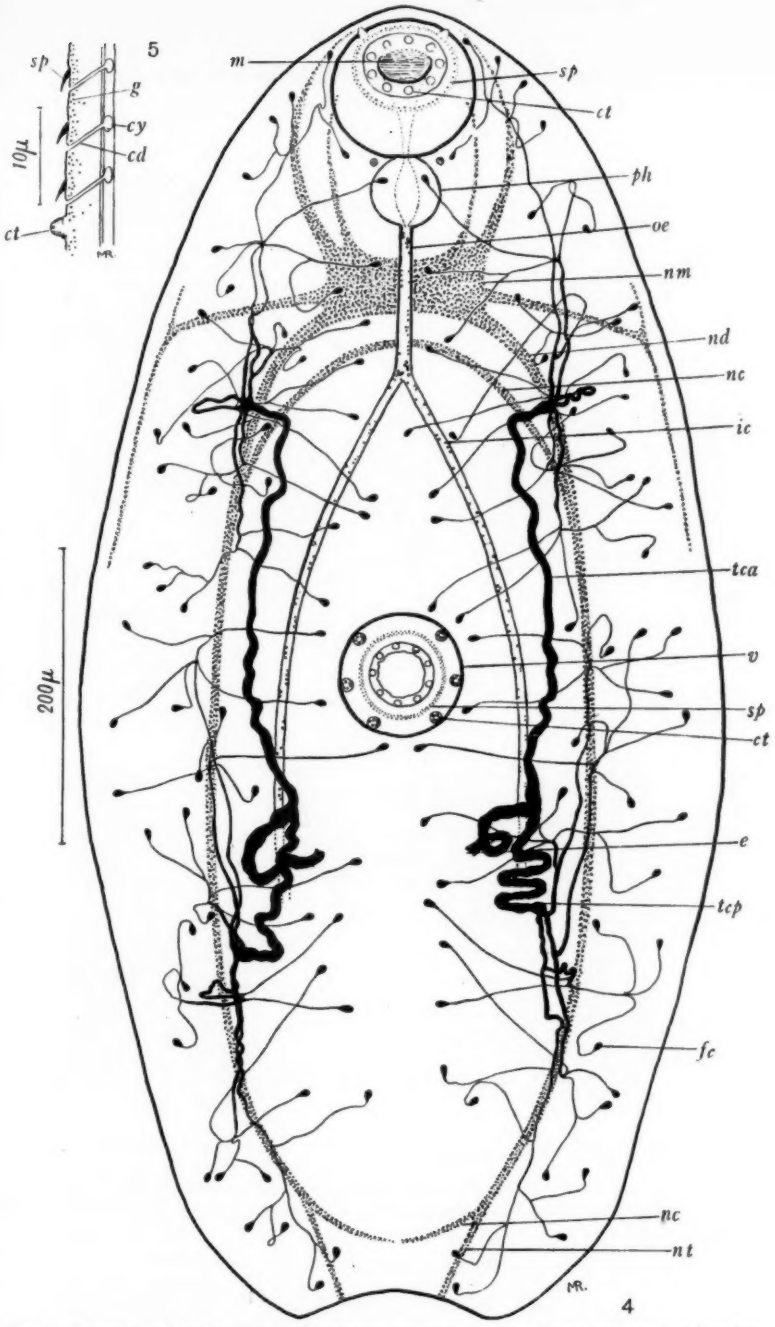


Fig. 4. *Cercaria pythionike:* flame-cell pattern, nervous system, alimentary canal and suckers.
Fig. 5. *Cercaria pythionike:* optical section of cuticle.

For explanation of lettering see p. 169.

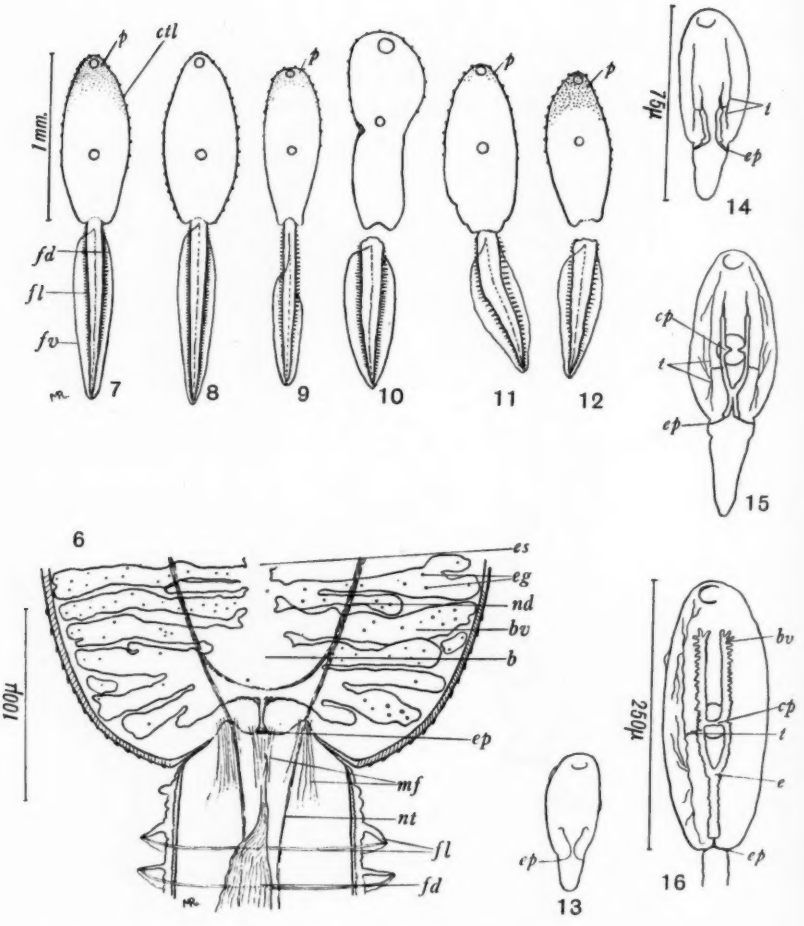


Fig. 6. *Cercaria herpsyllis:* bladder and anterior portion of tail.

Fig. 7. *Cercaria pythionike;* Fig. 8. *Cercaria nicarete;* Fig. 9. *Cercaria herpsyllis;* Fig. 10. *Cercaria doricha;* Fig. 11. *Cercaria ampelis;* Fig. 12. *Cercaria ranzii:* typical death attitude under a coverslip.

Figs. 13–16. *Cercaria pythionike:* development of excretory system.

For explanation of lettering see p. 169.

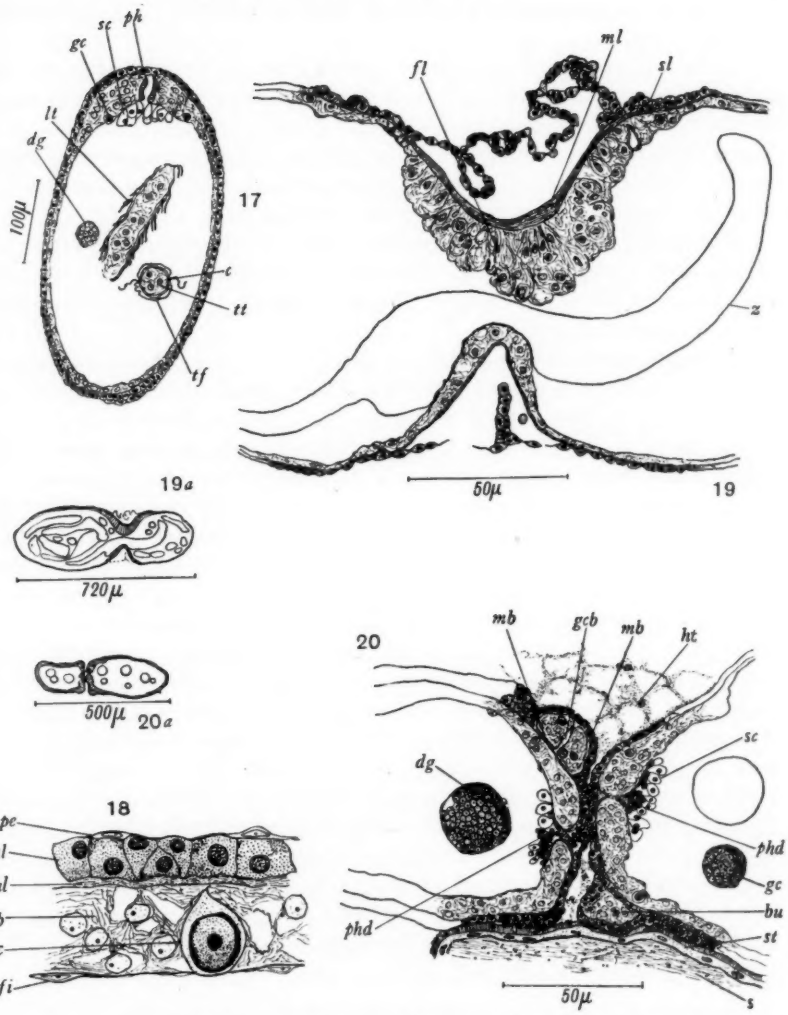


Fig. 17. *Cercaria pythionike:* longitudinal section of the sporocyst of second daughter generation, containing section of tail of developing cercaria.

Fig. 18. Section through body wall of young sporocyst.

Fig. 19. *Cercaria pythionike:* longitudinal section through constricting portion ("collar") of dividing sporocyst.

Fig. 19*a*. Outline drawing of the whole sporocyst of Fig. 19.

Fig. 20. *Cercaria pythionike:* longitudinal section (*in situ*) of approximated portions of recently divided sporocyst, showing developing pharynx, secretory cells, and two buds.

Fig. 20*a*. Outline drawing of the whole sporocyst of Fig. 20.

For explanation of lettering see p. 169.

Circle of six, each garnished with six small protuberances, on outer lip of ventral sucker.

Numerous granules, staining deeply with neutral red, embedded in surface of cuticle. Absent from lips of suckers. Most numerous posteriorly, floating out if cuticle ruptures. Function unknown.

In addition entire surface of body, except suckers, covered with backwardly projecting spines arranged in longitudinal rows. Small anteriorly, increasing in size posteriorly. Tail devoid of armature.

Oral sucker subterminal, surrounding mouth. Circle of minute closely set spines, three deep, on outer lip. Ventral sucker slightly smaller, situated a little below middle of body length. Protrudes slightly from surface. Possesses circle of spines, two deep.

*Excretory system and its development* (Figs. 1, 4, 13–16). Dilatation, present at base of stem of Y-shaped excretory vesicle, can be termed the bladder. Stem forks well below ventral sucker and the two branches pass upwards on either side of it, to anterior extremity. Joined by a cross-piece immediately below ventral sucker. Main lateral branching diverticulae on outside of vesicle number between thirty-five and forty-five. On inner side many irregular little evaginations present. Excretory pore situated at posterior end of body. Communicates with terminal portion of bladder by short narrow tube supplied with powerful sphincter muscle.

Main collecting tubes leave vesicle immediately posterior to ventral sucker. After short winding course, divide into antero- and postero-lateral collecting tubes each receiving canaliculi from thirty flame cells. Midway between the suckers the antero-lateral collecting tube dilates and splits into five branches. Two continue anteriorly, the inner one giving off one minor branch. Two others pass in backward direction towards ventral sucker. The fifth is coiled upon itself. Each branch terminates in group of five flame cells. Postero-lateral collecting tube dilates and splits into four branches well below level of "cross-piece". Three pass anteriorly to region of ventral sucker. One runs to extreme posterior end of body giving off one short branch below its point of origin and another half-way along its course. Branching often irregular (Fig. 4). All six branches terminate in group of five flame cells.

Flame-cell pattern (Fig. 4):

$$2\,[(5+5+5+5+5+5+5)+(5+5+5+5+5+5+5)]=120.$$

Two abnormal specimens found with six flame cells in most posterior group of antero-lateral collecting tube. Refractile granules present in small numbers. Largest in anterior region measuring up to 9·5$\mu$ in diameter, breaking down into smaller granules posteriorly.

Excretory system develops as two separate canals each originating at the point of the future division of body and tail. Proceeding anteriorly dividing into antero- and postero-lateral collecting tubes. Later the vesicle is (first) seen as dilatation of tubes, slightly above their posterior extremity. Dilated

portions gradually extend anteriorly, converging at point where branches of future **Y** meet. They diverge again passing upwards. A lateral swelling appears on inner side of each canal, in region of ventral sucker, which afterwards meet to form "cross-piece" of **Y**. Cross-piece forms before any lateral evaginations occur.

As cercaria develops, dilated lower portions of excretory tubes fuse to form bladder and stem of **Y**. Extreme posterior ends remain separate for considerable time afterwards. Lateral evaginations develop irregularly and slowly along edges of vesicle attaining full size shortly before cercaria emerges.

*Alimentary canal* (Fig. 4). Mouth leads into well-developed pharynx, followed by medium-sized oesophagus, which divides into two narrow intestinal caeca reaching posterior end of body. Caeca transparent; in specimens with developed penetration and cystogenous glands practically invisible.

*Penetration glands* (Fig. 1). One compact mass of elongated lobed cells, situated immediately posterior to bifurcation of oesophagus, and second more straggling group posterior to these, and behind ventral sucker. All stain brightly with eosin in mounted preparations. Cells of anterior group stain deeply with neutral red. Cells of posterior group scarcely stain at all and large nucleus sometimes visible.

Ducts narrow and undulating, following course of oesophagus for some distance, and then dividing into four groups. Two inner groups, which seem to contain all ducts of posterior, as well as some of anterior penetration-gland cells, pass on either side of pharynx, behind mouth, and bend sharply forward to open on anterior lip of sucker by about twenty-four closely set refractile apertures. External groups, containing about ten ducts each, pass round outside edges of sucker, bending inwards to open on anterior lip, more or less in line with apertures of central group. Ducts from eight most centrally situated glands of anterior group of cells pass out beneath these lateral ducts, and open, four a side, on ventral surface on either side of sucker (forty-two ducts in all).

*Nervous system* (Fig. 4). Central nervous mass lying under pharynx. Gives rise to following nerves:

Pair of lateral nerve cords from posterior lateral edges running parallel to intestinal caeca to end of body where they join. Large bundles of nerve fibres from each pass to tail. Pair of nerves from middle lateral edges, passing outwards to lateral edges of body, where they split into ascending and descending branches. Two pairs of nerves from anterior lateral extremities which pass upwards round oral sucker.

A commissure, also dorsal, unites main nerve cords above bifurcation of oesophagus.

*Reproductive system.* A group of deeply staining cells immediately above ventral sucker is probably the anlage of cirrus sac, and associated structures. Apart from these no traces of reproductive organs visible.

*The cystogenous glands* (Fig. 5). Are embedded immediately below the surface of cuticle, scattered thickly over surface of body. Open by slanting ducts into small pits on surface. Apertures refractile.

*Behaviour.* The cercariae emerge from the host singly or in pairs at the rate of two a minute. In the laboratory, on leaving the host they rise directly to the surface of the water where they remain for fifty hours and swim actively. The body is bent ventrally and driven through the water by vigorous lashings of the tail. After reaching the surface, short periods of activity (about 30 sec.) alternate with short periods of passive sinking through the water. In the latter condition, and when at rest on the bottom, the cercaria is bent in a characteristic **U**-shaped figure.

During the third day the cercariae sink to the bottom of the jar where they remain until the seventh or eighth day when death occurs.

When the cercariae, immediately on emerging from the snail, were placed in the bottom of a glass tube 30 ft. high, filled with sea water, they swam upwards until the top was reached in about 5 hours, when the up and down movements were resumed after contact with the surface.

Decaudation does not take place readily, but if severed from the body the tail swims actively in an **S**-shaped figure. The body is incapable of much independent movement.

A sudden rise in the temperature of the water generally induces an emission of cercariae from the host, sometimes before the cells in the tail have disintegrated. They rarely emerge unless the temperature of the water rises to 15° C. Two infected *Turritella* were kept nine weeks at a temperature below 8° C. and no cercariae emerged.

On dissection the sporocysts were found to be much longer than usual (*i.e.* than in *Turritella* kept at temperatures about 12–15° C.) and contained large numbers of moribund cercariae in an advanced state of development.

The cercariae are phototactic and swim gradually away from the source of light. Their reactions become more marked on the second day after emergence.

The presence of nerve fibres in the tail suggested testing them for reaction to light. A large number of tails were removed from the bodies of cercariae but, though swimming actively, they showed no phototactism.

If a number of cercariae are resting on the bottom of a shallow dish or on a slide a sudden increase in light produces instant activity.

A touch with a needle or pipette, while a cercaria is active, will induce a resting phase often prolonged for a considerable period.

*Parthenita* (Figs. 3, 17–20*a*). (The following description applies to all 6 species.)

Average infection 200–400 sporocysts. Seat of infection interlobular spaces of gonads of host (secondarily attack digestive gland). Very closely packed and entangled but not coiled. Outer layer of body wall applied to seminiferous tubules which are often reduced to mere bright orange threads. Size from $87 \times 50\mu$ to $2 \times 0{\cdot}5$ mm., sausage-shaped tapering slightly anteriorly. Colour yellowish grey to deep orange, brightest at both ends where body wall is thickest (Fig. 3).

Poorly developed pharynx situated anteriorly similar to that described by

E. C. Faust (1921) for *Cercaria pekinensis*, but surrounded by secretory cells resembling "salivary" glands of rediae. Pharynx acts also as birth pore.

Ovary "diffuse". Germ cells more numerous anteriorly. Fertility high. Large sporocysts contain 30–40 fully developed cercariae.

Body wall (Fig. 18) consists of five layers (characteristic of rediae, ex. *Cercaria saggitarius* Sinitzin 1911), thickened anteriorly to depth of $37\mu$, but towards centre dwindling to less than $4\mu$, where cells are flattened and nuclei lenticular.

(1) *First layer of body wall. Primitive epithelium* (*most external layer*). One cell thick. Flattened cells with ill-defined boundaries and discoid nucleus, $1{\cdot}8 \times 3{\cdot}7\mu$. Well-defined nuclear membrane; several nucleoli. Poorly staining cytoplasm.

(2) *Second layer.* Large non-vacuolated cells, $7 \times 5\mu$ to $3 \times 3\mu$, with well-defined cell wall, deeply staining protoplasm and nucleus. Nucleus spherical or ovoid, with well-defined nuclear membrane. Chromatin network with large accumulations of chromatin at points of intersection. No nucleoli. This layer and fourth layer are the principal components of the body wall. Often several cells deep, particularly anteriorly. It contains scattered pigment granules, and is easily detachable, as single sheet of tissue, from rest of the sporocyst.

(3) *Third layer.* Muscle fibres.

(4) *Fourth layer.* Large vacuolated eosinophil cells. Single spherical nucleus not deeply staining. Nuclear membrane distinct. Fine chromatin network. Single large nucleolus. These cells are $20\mu$ deep in anterior region of sporocyst but the middle of body becomes flattened with no visible boundaries and protoplasm reduced to threads. Refractile granules are present in small aggregations.

*Germ cells.* Large spherical compact cells occurring throughout this layer but far more numerous anteriorly. Nucleus perfectly spherical with very distinct nuclear membrane and single deeply staining nucleolus. Protoplasm is reduced to a narrow rim. Sometimes attached by peduncle to base of the layer. Measurements with peduncle $14 \times 11\mu$. Nucleus $9\mu$ in diameter.

(5) *Fifth layer* (*most internal*). One cell deep. Flattened cells with ill-defined boundaries and poorly staining single lenticular nucleus $7 \times 1{\cdot}8\mu$. One nucleolus. Nuclear membrane present but chromatin network poorly defined.

*Reproduction of parthenita.* In young stages (not exceeding $150\mu$) the sporocysts appear to have no pharynx or birth pore, the body is oval with a body wall $14–18\mu$, and granules crowded together.

At this stage two types of division take place: (*a*) the formation of minute buds at both extremities (see Fig. 20); (*b*) transverse fission, by a central constriction, which is followed by division (see Fig. 19*a*).

The severed ends of the sporocyst invaginate and form the future "pharynx", round which the secretory cells develop.

Neither type of division has been noted when the sporocysts are full grown and have not emitted cercariae. It occurs again vigorously, after a large emission which leaves the sporocysts ragged and collapsed. Thus a second

generation of daughter sporocysts arises, which are much smaller, often containing only one or two nearly full-grown cercariae. Thus the degree of fertility of these sporocysts depends on the age of the infection. This character was wrongly taken by many authors as specific. In this case division often occurs when one or two full-grown cercariae are still present (see Fig. 19). Short chains of sporocysts are sometimes formed (as noted for other species by Harper, 1929; Dubois, 1929; Mathias, 1925) by other constrictions forming before the first severance takes place.

The fourth layer of the body wall plays the greatest part in division, growing inwards to form a collar 25–30$\mu$ deep (see Fig. 19).

The only specific differences observed between the sporocysts are the number and size of the granules in the body wall. In *Cercaria pythionike* the granules are arranged in scattered groups, the maximum diameter being 2$\mu$.

The sporocysts apparently develop slowly. A *Turritella*, kept in plunger jars in London for six months, showed on dissection a very young infection. The undamaged state of the gonad and digestive gland of the host proved this infection to be a first generation of daughter sporocysts. This factor adds to the difficulty of work on the life history.

**Cercaria herpsyllis** n.sp. (Figs. 6 and 9)

Measurements in microns

| | |
|---|---|
| Body length ... ... ... ... ... ... | 800–640 |
| Body width (maximum) ... ... ... ... | 400–240 |
| Tail length ... ... ... ... ... ... | 960–800 |
| Tail width (maximum without fins) ... ... ... | 95– 85 |
| Lateral fins depth (maximum) ... ... ... | 80– 48 |
| Dorsal fin width (maximum) ... ... ... ... | 96–100 |
| Ventral fin width (maximum) ... ... ... | 96–100 |
| Oral sucker ... ... ... ... ... ... | 80 × 80 |
| Ventral sucker ... ... ... ... ... ... | 80 × 70 |
| Pharynx... ... ... ... ... ... ... | 27 × 22 |
| Oesophagus (width) ... ... ... ... ... | 7–6 |
| Oesophagus (length) ... ... ... ... ... | 28 |
| Excretory granules (maximum) ... ... ... | 3·4 × 3·4 |
| Sporocyst granules (maximum) ... ... ... | 2·4 × 2·4 |

0·5 *per cent. infection.*

Smallest known British species of *Rhodometopa* group. Pink pigment present in anterior end of body. Excretory granules fairly numerous and up to 3·4$\mu$ in diameter.

Dorsal and ventral fins small and under coverslip do not reach beyond edges of lateral fins until about half-way down the tail (Fig. 9). In other species they overlap almost from point of origin. Penetration-gland ducts larger with dilatations along their course. Both glands and ducts appear more coarsely granular. Minute granules can sometimes be seen moving in dilated portions near the apertures. Glands of posterior group behind ventral sucker do not stain at all with neutral red and are exceedingly difficult to see.

Stem of main excretory vesicle (Fig. 6) (without lateral evaginations) narrower and "arms" of the Y wider. Cross-piece fuses later in development. Flame-cell pattern shows up with comparatively great clarity. Alimentary canal can barely be detected in fully developed specimens.

If the cercaria is observed under the binocular microscope the outlines of the anterior branches of the main excretory vesicle cannot be seen. Their presence can be detected by the granules which partially fill them. In *Cercaria pythionike* and *C. nicarete* the outline is clearly visible and only one or two of the maximum-sized granules are present in them. Thus in *C. herpsyllis*, *C. doricha* and *C. ampelis* the anterior end of the excretory vesicle appears darker instead of lighter than the rest of the body.

*Parthenita.* Granules in sporocysts body wall measure up to 2·4 $\mu$ in diameter.

It is interesting to note that the largest sporocyst granules are found in those species which possess the smallest excretory granules in the cercaria stage.

**Cercaria doricha** n.sp. (Figs. 2 and 10)

Measurements (relaxed under a coverslip) in microns

| | |
|---|---|
| Body length | 1411 |
| Body width opposite ventral sucker | 531 |
| Tail length | 1328 |
| Tail width at widest without fins | 99 |
| Lateral fins (maximum depth) | 88 |
| Dorsal fin (maximum) | 150 |
| Ventral fin (maximum) | 150 |
| Oral sucker | 116 × 116 |
| Ventral sucker | 99 × 99 |
| Anterior end to border of ventral sucker | 638 |
| Pharynx | 37 × 46 |
| Oesophagus | 74 × 13 |
| Excretory granules (maximum) | 2·9 × 2·9 |
| Flame cell | 7·4 |
| Sporocyst granules (maximum) | 5·2 × 5·2 |

Measurements (at rest without a coverslip) in microns

| | |
|---|---|
| Body length | 1488 |
| Body width opposite ventral sucker | 560 |
| Tail length (which is then contracted) | 562 |
| Tail width (maximum) with fins | 160 |
| Oral sucker | 80 × 80 |
| Ventral sucker | 64 × 64 |

5 *per cent. infection.*

The largest species, easily picked out with the naked eye from other species in a plunger jar. More elongated, resembling a spear-head in outline. Tail shorter than body. Colour opaque white. No pigment in body, but rarely present in tail (see p. 166).

Ventral sucker smaller in relation to oral sucker, situated above the middle of body length. Protruding very sharply from the surface.

Compared with *C. pythionike*: Cuticle more delicate. Neutral red penetrat-

ing considerably faster. Cuticular tubercles not so strongly developed, rarely persisting along lateral edges as far as middle of body.

Spines larger and more conspicuous.

Two lateral groups of penetration glands (in addition to both central groups) form elongated straggling masses parallel with oesophagus. Ducts follow contour of oral sucker and open on anterior lip. Central groups of glands also straggling with no clear differentiation between anterior and posterior groups. Latter stain with neutral red though not quite so intensely. Ducts from central groups follow the same course as in *C. pythionike*. Both gland cells and ducts are largest and most conspicuous in this species. Granulation more marked.

Lateral evaginations of main excretory vesicle more numerous and delicately branched. 50–60 large diverticulae on external lateral edges. Stem and branches of Y and cross-piece are narrower. Bladder bulb-shaped, wider in relation to stem. Excretory granules very numerous and small. Largest measure $3\mu$ in diameter.

Flame-cell pattern could only be partially worked out as the lateral groups of penetration glands obscure the anterior region. Branching of the postero-lateral collecting tube the same as in other species of the group but flame cells number six per group. It seems probable that the complete pattern would be: $2[(6+6+6+6+6+6)+(6+6+6+6+6+6)]=144$. An occasional specimen was found in which a group contained seven flame cells.

Cuticular granules smaller, not so numerous and appear to be arranged in rough circles round base of spines and openings of cystogenous cells.

Two infections of *C. doricha* were found in which 99 per cent. of the cercariae over $1000\mu$ in body length had pink pigment present in the tail.

*Behaviour.* The position adopted during the passive sinking period and when resting on the bottom is different from that of all other species of the group. The body is fully expanded and the tail is contracted and held stiffly in a line with the body. The cercaria sinks in a perpendicular line, tail uppermost. On the bottom it lies flatly, parallel with the surface and never on its side.

It is less active than the others and frequently sinks to the bottom where it remains quiescent for a considerable period before rising again to the surface. In the 30 ft. tube this species never rose above 9–10 ft. from the bottom.

Specimens removed from sporocysts, which are capable of swimming, behave somewhat similarly to *C. pythionike* when first freed in the water. After about thirty minutes they adopt their characteristic behaviour.

*Parthenita.* Diameter of maximum-sized granules of sporocyst wall $=5{\cdot}2\mu$.

### **Cercaria nicarete** n.sp. (Fig. 8)

1 *per cent. infection.*

Measurements identical with those of *C. pythionike* except that the excretory granules measure up to $11{\cdot}1\mu$ in diameter.

Characterised by more strongly developed cuticular tubercles along lateral

edges, conspicuous to posterior extremity of body. No pink pigment recorded from any part of cercaria. Body slightly more opaque than *C. pythionike.*

Sporocyst granules smallest recorded.

**Cercaria ampelis** n.sp. (Fig. 11)

Measurements in microns

| | |
|---|---|
| Body length ... ... ... ... ... | 1040–864 |
| Body width ... ... ... ... ... | 544–432 |
| Tail length ... ... ... ... ... | 880–720 |
| Tail at widest (without fins) ... ... | 144–128 |
| Tail with lat. fins (maximum width) ... | 244–240 |
| Oral sucker ... ... ... ... ... | 88 × 96–80 × 80 |
| Ventral sucker ... ... ... ... | 88 deep × 80–80 × 80 |
| Pharynx ... ... ... ... ... | 61 × 46 |
| Oesophagus ... ... ... ... ... | 38 long. |
| Longitudinal fins (maximum width) ... | 192–120 |
| Lateral fins (maximum depth) ... ... | 65–51 |
| Maximum-sized excretory granules ... | 3·7 × 3·7 |

4 *per cent. infection.*

The pink pigment in anterior end rather pale, often reduced to few specks of pink only visible under high power objectives. Body very transparent. Tail considerably shorter than in any of British species. Pharynx and intestine poorly developed only visible after prolonged staining with neutral red. Suckers of equal size. Ventral sucker practically in centre of body, if displaced posteriorly only by a few microns. Penetration glands very opaque, 34 ducts only. Lower group as in *C. herpsyllis.* Excretory vesicle filled with very numerous small granules measuring up to 3·7 $\mu$ in diameter making the vesicle dark on light background. Cuticular tubercles and spines moderately developed.

*Parthenita.* Granules in sporocyst wall very numerous, crowded together, measuring up to 1·8 $\mu$ in diameter.

**Cercaria ranzii**[1] n.sp. (Fig. 12)

Measurements in microns

| | |
|---|---|
| Body length ... ... ... ... ... | 780–640 |
| Body width (maximum) ... ... ... ... | 380–240 |
| Tail length... ... ... ... ... ... | 800–480 |
| Tail width (maximum without fins) ... ... | 128–128 |
| Tail width (maximum with lat. fins) ... ... | 240–224 |
| Oral sucker ... ... ... ... ... | 80 × 80–64 × 64 |
| Ventral sucker ... ... ... ... ... | 80 × 80–72 × 72 |
| Longitudinal fins (maximum width) ... ... | 144–128 |
| Pharynx ... ... ... ... ... ... | 27 long × 22 wide |
| Oesophagus (length) ... ... ... ... | 27 |
| Excretory granules (maximum)... ... ... | 11·1 × 11·1 |

1 *per cent. infection.*

Average size smallest of all species of group. Pink pigment in anterior end very bright.

Tail stout as in other species from Naples, being 33 $\mu$ wider than in *C. herpsyllis* which has about the same body size.

Excretory granules large as *C. nicarete,* very few in number. Excretory vesicle appears light on darker background.

[1] This species is named in honour of Prof. Silvio Ranzi.

Ventral sucker situated in middle of body line or a few microns above it. Ducts of cystogenous glands much finer than in other species from Naples and difficult to count, probably 34. Pharynx and oesophagus and intestine barely visible even after prolonged staining with neutral red. Cuticular protuberances do not reach $\frac{3}{4}$ of body length. Cuticle much tougher than in preceding species and outline remains more rigid under coverslip. Spines well developed.

*Parthenita.* Granules in body wall very crowded, small and variable in size.

### Life-history experiments

The following organisms were obtained in the dredge with the *Turritella* at Plymouth in September and December. The fish were trawled in the same vicinity.

| Species | Number |
|---|---|
| *Psammosolen chamasolen* (da Costa) ... ... | 21 |
| *Mysia undata* (Pennant) ... ... ... | 5 |
| *Clathrus clathrus* (L.) ... ... ... ... | 4 |
| *Odostomia eulimoides* (Hanley) ... ... | 11 |
| *Alpheus ruber* (Miln-Edwards) ... ... | 16 |
| *Upogebia stellata* (Montagu) ... ... ... | 32 |
| *Gonoplax rhomboides* (L.) ... ... ... | 58 |
| *Anapagurus laevis* (Thompson) ... ... | 4 |
| *Ebalia tuberosa* (Pennant) ... ... ... | 2 |
| *Crangon allmanni* (Kinahan) ... ... ... | 1 |
| *Ampelisca* sp. ... ... ... ... ... ... | 14 |
| *Dexamine spinosa* (Montagu) ... ... ... | 1 |
| *Leucothoë spinicarpa* (Abildgaard) ... ... | 1 |
| *Aphrodite* sp., *Melinna* sp., *Aricia* sp., *Notomastus* sp., *Glycera* sp., *Nephthys* sp. — Several of each | |
| *Phascolion strombi* (Montagu) ... ... | 15 |
| *Echinocardium* sp. ... ... ... ... | 1 |
| *Astropecten irregularis* (Pennant) ... ... | 2 |
| *Asterias rubens* (L.) ... ... ... ... | 1 |
| *Cucumaria elongata* (Düben and Koren) ... | 138 |
| *Thyone raphanus* (Düben and Koren) ... | 3 |
| *Leptosynapta inhaerens* (Müller) ... ... | 10 |
| *Gadus pollachius* (L.), *Gadus luscus* (L.), *Gadus merlangus* (L.), *Pleuronectes platessa* (L.), *Pleuronectes limanda* (L.), *Solea vulgaris* (Quensel), *Solea variegata* (Donovan), *Lophius piscatorius* (L.), *Trigla* sp., *Cepola rubescens* (L.), *Callionymus lyra* (L.), *Rhina squatina* (L.), *Raja maculata* (Montagu) — Several of each | |

It seemed unlikely from a study of the behaviour of the cercariae that the

second intermediate host would be found living continually on the sea bottom. The search for metacercariae was fruitless and all infection experiment failed.

Two specimens of *C. doricha* made abortive efforts at boring into the fins of *Pleuronectes platessa* (L.). The cercaria balanced on the anterior sucker, and body and tail were thrown suddenly into a corkscrew shape. None achieved penetration. So far the evidence suggests that the second intermediate host may be a fish.

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**Explanation of Lettering**

| | |
|---|---|
| *b* | Bladder. |
| *bp* | Birth pore. |
| *bu* | Bud. |
| *bv* | Branch of excretory vesicle. |
| *c* | Caudal cells. |
| *cd* | Cystogenous gland duct. |
| *cp* | "Cross-piece" of excretory vesicle. |

| | |
|---|---|
| *ct* | Cuticular tubercles. |
| *ctl* | Lateral cuticular tubercles. |
| *cy* | Cystogenous gland. |
| *d* | Ducts of penetration glands. |
| *dg* | Developing germ ball. |
| *e* | Excretory vesicle. |
| *eg* | Excretory granules. |
| *ep* | Excretory pore. |
| *es* | Stem of excretory vesicle. |
| *ev* | Point of entry of collecting tube into main excretory vesicle. |
| *fb* | Fourth layer of sporocyst body wall. |
| *fc* | Flame cell. |
| *fd* | Dorsal fin. |
| *fi* | Fifth layer of sporocyst body wall. |
| *fl* | Lateral fin. |
| *fv* | Ventral fin. |
| *g* | Granules in cuticle. |
| *gc* | Germ cell. |
| *gcb* | Germ cell developing in bud. |
| *ht* | Tissue of host. |
| *ic* | Intestinal caeca. |
| *lt* | Longitudinal section of part of tail. |
| *m* | Mouth. |
| *mb* | Muscular layer of bud. |
| *mf* | Muscle fibres. |
| *ml* | Muscular layer of sporocyst body wall. |
| *nc* | Nerve commissure. |
| *nd* | Main dorsal nerve cord. |
| *nm* | Central nerve mass. |
| *nt* | Nerve to tail. |
| *o* | Oral sucker. |
| *oe* | Oesophagus. |
| *p* | Pink pigment. |
| *pa* | Apertures of penetration glands. |
| *pe* | Primitive epithelium or outmost layer of sporocyst body wall. |
| *ph* | Pharynx. |
| *phd* | Developing pharynx. |
| *pl* | Lateral group of penetration glands. |
| *pm* | Median group of penetration glands. |
| *r* | Refractile granules. |
| *s* | Spermatozoa of host. |
| *sc* | Secretory cells. |
| *sl* | Second layer of sporocyst body wall. |
| *sp* | Spines. |
| *st* | Seminiferous tubules of host. |
| *t* | Collecting tubes. |
| *tca* | Lateral collecting tube (anterior). |
| *tcp* | Lateral collecting tube (posterior). |
| *tf* | Tail fins. |
| *tt* | Transverse section through tail. |
| *v* | Ventral sucker. |
| *x* | Position of dorsal fin when flattened under a coverslip. |
| *xx* | Position of ventral fin when flattened under a coverslip. |
| *y* | Yellow pigment. |
| *z* | Outline of developing cercaria caught between constriction. |

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# NOTE ON THE EXCRETORY SYSTEM OF *CERCARIA EPHEMERA* LEBOUR, 1907 (*NEC* NITZSCH)

By MIRIAM ROTHSCHILD

(With 7 Figures in the Text)

*Cercaria ephemera* Lebour belongs to the group of Monostome cercariae which have been proved to belong to the Notocotylidae. This species probably belongs to the genus *Notocotylus*.

When the cercaria leaves the snail host to encyst in a free state upon the shell, or other objects, the cystogenous glands are so packed with refringent material that the study of the details of the excretory system is practically impossible.

This character probably accounts for the fact that only two descriptions of the whole excretory system of species of this group have been published. Faust (1919) described a Monostome cercaria, *C. spatula*, which he regarded as probably belonging to the Notocotylidae.

Horsfall (1931) described a typical Monostome of this group, *C. infracaudata*, possessing a similar type of excretory system to *C. spatula*.

The excretory system in *C. ephemera* Lebour, in very early stages, consists as in *C. spatula* of two parallel canals, arising in the posterior end of the body (Fig. 1). Each grows forward to the future oesophageal region and then turns back. This descending portion forms the main collecting tube. It is terminated by a single flame cell which was not observed beating. In the posterior region of the body the parallel canals swell, draw together and coalesce to form the bladder. A very marked thickening occurs in that portion of the canals, which ultimately become the main excretory vesicle.

At a little later stage the canals in the tail portion coalesce (Fig. 2) (later fusing to form the median caudal excretory tube), and the anterior portions of the main excretory vesicle can be seen growing towards each other in the region of the oesophagus. Here they eventually join, thus completing the "tubular circuit".

At this time each main excretory tube lies well to one side of the excretory vesicle, reaching to about half its length (Fig. 2), where a bifurcation into an ascending and descending branch takes place, with a flame cell at each extremity.

The anterior branch now divides into three flame cells. In view of Sewell's theory (1922) of the relationship between the "Ephemera" group of Monostomes and the "Pigmentata" group of Amphistomes, it is interesting to note that it is the anterior daughter flame cell which divides first.

By the time the tubular circuit is clearly completed, each collecting tube has shifted inwards and comes to lie directly below the parallel portions of the main vesicle (Fig. 3).

The posterior daughter flame cell also divides into three, and a fourth flame cell is given off by both anterior and posterior branches close to their

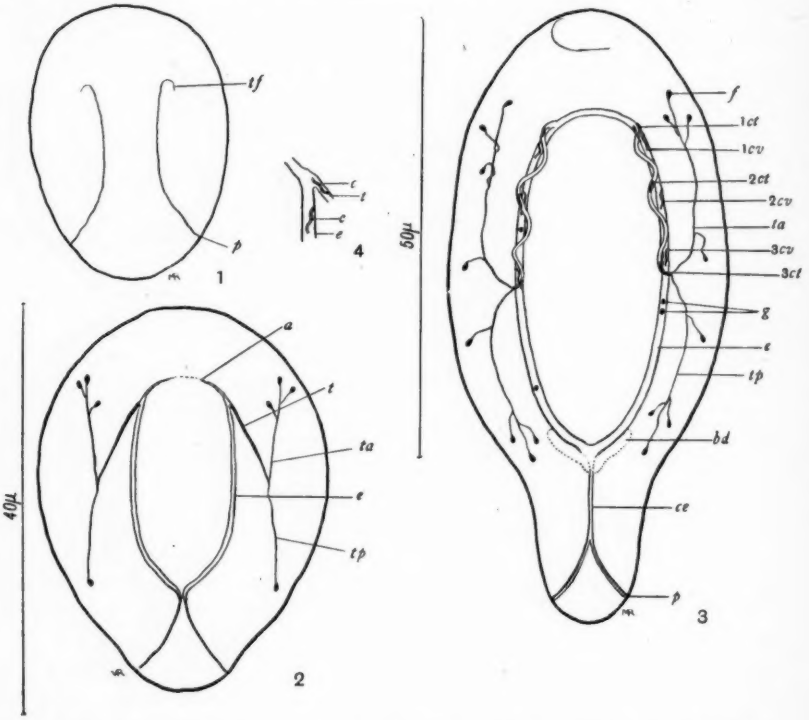


Fig. 1. Development of the excretory system of *C. ephemera* Lebour, showing parallel ducts.
Fig. 2. Development of the excretory system of *C. ephemera* Lebour, showing tubular circuit about to be completed.
Fig. 3. Development of the excretory system of *C. ephemera* Lebour, showing the bladder developing and flame cell pattern.
Fig. 4. Juncture of excretory vesicle and collecting tube.

original bifurcations (Fig. 3). The fourth flame cell is definitely isolated from the terminal groups. It seems possible that it splits off from the main branches at an earlier stage. The flame cell pattern, which is $2[(3+1)+(3+1)]=16$, agrees with the pattern of the full-grown *C. infracaudata*.

In addition to the flame cells, six bundles (each side) of actively beating cilia (Fig. 3) are situated in the following positions:

(*a*) Attached to the wall within the main collecting tube: (1) At the division into the anterior and posterior collecting tube. (2) Midway between

this division and the juncture with the main excretory vesicle (Fig. 3). (3) At the juncture of the main collecting tube and the main excretory vesicle (Figs. 3, 4).

(*b*) Attached within the anterior half of the main excretory vesicle on the lateral (outer) wall: (1) Below the juncture of the vesicle and the main excretory tubes. (2) About half-way along the parallel portion of the vesicles. (3) At equal distance between (1) and (2).

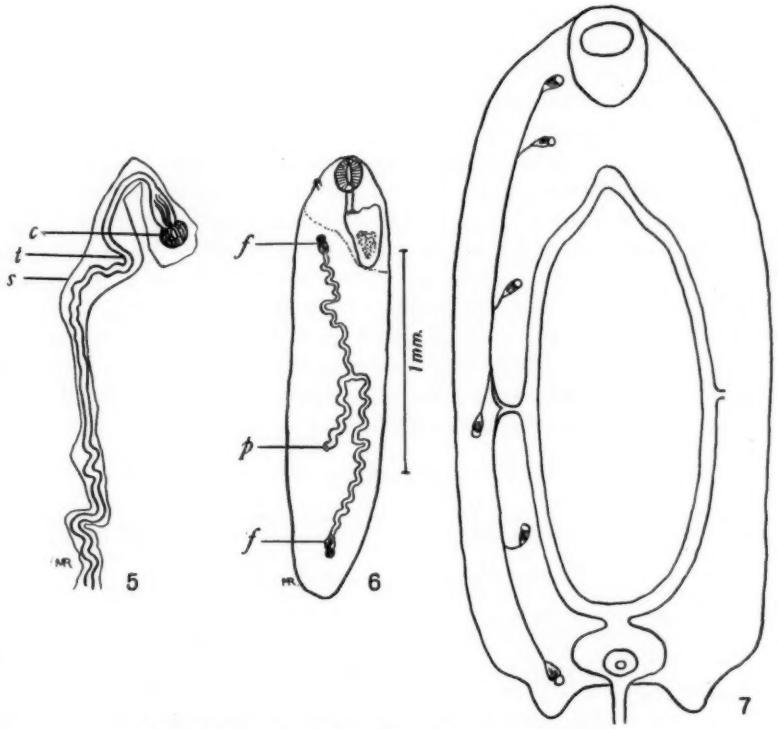


Fig. 5. Flame cell and duct of redia within excretory sinus.
Fig. 6. Excretory pattern of redia.
Fig. 7. Excretory system of *C. spatula* Faust (after Faust).

The cilia can be observed beating soon after the bifurcation of the collecting tubes. Although in the early stages there are only a few granules present, later, when the vesicle becomes closely packed, the beating of the cilia must be greatly impeded, if not totally abolished.

Unfortunately early development of the cystogenous glands prevents any further count of flame cells, but an occasional glimpse of one in an unusual position suggests that the pattern becomes more complicated by the "fourth" flame cell dividing again.

The excretory system of the redia (Fig. 6) is very similar to that of *C. spatula*. A single large flame cell is situated in the anterior and posterior end of the body, and the two collecting tubes open into a short common excretory duct terminating in an excretory pore in the middle region of the body (on each side of the body). The excretory sinus is clearly visible (Fig. 5).

From the above description it will be seen that *C. ephemera* Lebour displays the typical "Stenostoma" type of excretory system as anticipated by Sewell (1922) and, unlike *C. spatula*, is devoid of unusual features. In the latter species (and in *C. robusta* and *infracaudata* (?)) the anterior portions of the main collecting tubes seem to have been suppressed. In *C. spatula* the single anterior flame cell on each side of the body "wanders backwards along the lateral tubules" and comes to occupy "a place half-way between the anterior end of the system and the bladder", the main collecting tube thus entering the vesicle half-way along its length (Fig. 7). Thus full-grown cercariae have developed an excretory system which resembles the arrangement in the "Mesostoma" type, when, as is sometimes the case (Dollfus, 1930; Rothschild, 1935), the main excretory vesicle continues growing anteriorly after the development of the main collecting tubes. Only careful study of the early stages reveals that it is fundamentally of the "Stenostoma" type.

Owing to the difficulty of tracing the finer vessels, this description appears to need corroboration, particularly as the excretory system of a closely related form such as *C. ephemera* Lebour conforms strictly to the "Stenostoma" type and does not show this peculiar modification.

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**Explanation of Lettering**

*a*, anterior portion of excretory tubes fusing to form tubular circuit; *bd*, developing bladder; *c*, cilia; *ce*, caudal excretory tube; 1 *ct*, first bundle of cilia in main collecting tube; 2 *ct*, second ditto; 3 *ct*, third ditto; 1 *cv*, first bundle of cilia in excretory vesicle; 2 *cv*, second ditto; 3 *cv*, third ditto; *e*, excretory vesicle; *f*, flame cell; *g*, excretory granules; *p*, excretory pore; *s*, excretory sinus of redia; *t*, main collecting tube; *ta*, anterior collecting tube; *tf*, future main collecting tube; *tp*, posterior collecting tube.

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# A REDESCRIPTION OF *TAENIA TENUICOLLIS* RUDOLPHI, 1819, AND ITS LARVA, *CYSTICERCUS TALPAE* RUDOLPHI, 1819

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(With 2 Figures in the Text)

This is the first report of *Taenia tenuicollis* Rudolphi, 1819, and its larva, *Cysticercus talpae* Rudolphi, 1819, from North America. In the process of identification of the specimens described below, it became evident that no adequate figures of this species had been published, that this specific name had been given by recent authors to more than one species of Cestode, and that several characters of value in specific differentiation had not been described. The description given here is based entirely upon specimens from *Mustela vison* collected by Dr R. G. Law of the Experimental Fur Farm, Kirkfield, Ontario. Details published by Thienemann (1906) and others on the European specimens of this species are given in the accompanying comparative table.

## **Taenia tenuicollis** Rudolphi, 1819

Synonyms: (?) *Taenia serrata* Goeze, 1782, of Rudolphi, 1810; (?) *T. pusilla* Goeze, 1782; (?) *T. mustelae* Gmelin, 1790; (?) *Halysis putorii* Zeder, 1803; (?) *H. mustelae* (Gmelin 1790) Zeder, 1803; (?) *Taenia mustelae vulgaris* Rudolphi, 1810; (?) *T. putorii* (Zeder 1803) Rudolphi, 1810; "*T. intermedia*" of Cameron, 1933.

Discussion of the synonymy is given in the general discussion of this species.

*Specific differentiation.* On the basis of the following characters this species may be separated from other members of the genus: Worms of relatively small size, length of known specimens from North America not exceeding 7 cm. Suckers relatively large. Rostellar hooks smaller than those from other known species from carnivores, length not exceeding 16 $\mu$. Genital papillae relatively prominent, with well-developed musculature, situated in anterior half of segment margin in mature segments but usually in middle of margin in gravid segments. Genital atrium relatively large, its shape depending upon state of contraction of segment. Vagina showing several serpentine coils in regions between lobes of ovary and median portion of segment just anterior to ovary; vagina frequently showing an increase in diameter just before entering genital atrium; walls of vagina lined throughout with ciliated cells. Seminal receptacle between lobes of ovary. Cirrus pouch conspicuous, usually ovoid in contracted segments. Cirrus composed of two portions, the outer (next to genital atrium)

much larger in diameter and lined with ciliated cells, the inner of smaller diameter and without cilia (Fig. I, 2). Ventral excretory vessels conspicuous, usually showing great increase in diameter at point of union with transverse excretory vessels. Contracted mature segments in the six specimens available for study show a uniformity in "topography". Vitellarium never extending laterally beyond ovary. Detailed description of these characters is given in the following paragraphs:

| | European specimens of *Taenia tenuicollis* as described by Thienemann (1906) and others | Specimens of *Taenia tenuicollis* from Kirkfield, Ontario |
|---|---|---|
| Length | 12–147 mm. | Up to 70 mm. |
| Width | Up to 2 mm. | Up to 2·4 mm. |
| Neck* | 1·9–2·2 mm. long | 28 $\mu$ to 1 mm. long |
| Scolex | 260–550 $\mu$ wide | 237–303 $\mu$ wide |
| Hooks (total number) | 36–72 | About 42† |
| Small hooks‡ | 13–21 $\mu$ long | About 15–16 $\mu$ long§ |
| Large hooks‡ | 16–24 $\mu$ long | About 15–16 $\mu$ long§ |
| Genital papillae | Prominent, usually anterior to middle of segment | Prominent, usually anterior to middle of segment |
| Excretory canals | Conspicuous | Conspicuous |
| Testes (number) | 60–114 | 90–125 |
| Testes (diameter) | 42–67 × 37–52 $\mu$ | 39–55 $\mu$ |
| Uterine branches | 12–18 | 10–19 |
| Eggs (diameter) | 20–23 × 24–28 $\mu$ | 17–20 $\mu$ |
| Hosts (definitive) | ‖ *Mustela putorius* (syn. *Foetorius putorius*), *M. nivalis* (syns. *M. vulgaris*, *Foetorius vulgaris*, *Putorius vulgaris*, *Arctogale nivalis*), *Mustela erminea* (syn. *Arctogale erminea*) | *Mustela vison* |
| Hosts (intermediate) | ‖ *Cricetus cricetus* (syn. *C. frumentarius*), *Talpa europaea*, *Microtus agrestis*, *M. a. hirtus*, *M. arvalis* (syns. *Arvicola arvalis*, *Lemmus arvalis*), *Apodemus sylvaticus* (syn. *Mus. sylvaticus*), *Clethrionomys glareolus* (syn. *Evotomys glareolus*), *C. rufocamus* (syn. *Evotomys rufocamus*), "field mouse", white mouse (experimental) | *Ondatra zibethica*, *O. spatulata* |

* The most anterior segments are sometimes much narrower than posterior immature segments thus giving the impression, upon superficial examination, of a very long neck. In measuring the neck region, the present writer included only the portion of the strobila showing no traces of segmentation.

† In the larva, *Cysticercus talpae*, the number of hooks is 42–60.

‡ Thienemann (1906) recorded the length of the small hooks as 0·01386 (0·02) mm. [*sic*] and the length of the large hooks as 0·01638 mm.

§ Accurate measurement is impossible with the limited material of the adult Cestode. Hooks are apparently deeply embedded in tissues of rostellum and easily broken, since they disintegrated in one rostellum before it was possible to separate them in order to secure a lateral view. Large hooks of *Cysticercus talpae* from Canada are 16–20 $\mu$ long, small hooks 16–18 $\mu$ long.

‖ Dr Gerrit S. Miller, Jr., of the National Museum has given the synonymy used in this list of mammalian hosts.

*Scolex.* Diameter at suckers 237–303 $\mu$ (Fig. I, 3). Suckers relatively large, 77–110 $\mu$ wide, usually slightly longer than wide. Rostellum 61–77 $\mu$ in

diameter, frequently extruded as a globular structure about 66 $\mu$ in diameter. Hooks about 48 in number, usually lost, present in only two specimens, taenioid in shape, arranged in two circlets; hooks of both rows apparently similar in size and shape, about 15–16 $\mu$ long, guard of hooks prominent and apparently bifid. No accurate measurements and no drawing of hooks could be made because of the difficulty of separating them to secure a lateral view; one scolex was sectioned in an attempt to secure a lateral view of an entire hook but without success. It is possible that hooks of posterior row are 2 or 3 $\mu$ shorter than those of anterior row, and that the hooks of both rows are somewhat longer than the measurements given here.

*General anatomy of strobila.* Length of specimens with gravid segments 5·1–7 cm., maximum width 2·4 mm. Neck 28 $\mu$ to 1 mm. long, length varying with contraction of neck region. About 68 immature segments; mature segments few in number, about 5–10; about 14–25 gravid segments. Length-width ratio of segments varying with state of contraction of strobila; immature and mature segments usually broader than long; gravid segments usually longer than wide, up to 4·75 mm. long by 1·5 mm. wide. Genital papillae 127–275 $\mu$ long measured along longitudinal margin of segments, usually anterior to middle of segment margin. Genital atrium up to 175 $\mu$ in diameter; in relaxed gravid segments, 127–155 $\mu$ wide by 193–225 $\mu$ long. Nerve cord about 100 $\mu$ from lateral margin of segment. Calcareous corpuscles not observed.

*Male reproductive system.* Testes 90–125 in number, distributed chiefly in one layer, but sometimes overlapping dorso-ventrally; usually roughly spherical, 39–55 $\mu$ in diameter in relaxed segments; ovoid in contracted segments, 17–28 $\mu$ wide by 39–55 $\mu$ long; with usual distribution between longitudinal canals, rarely extending posterior to ovary lobes; no testes posterior to vitellarium. Cirrus pouch usually ovoid (Fig. I, 1), about 130–154 $\mu$ wide by 193–220 $\mu$ long. Cirrus usually contracted within pouch; composed of two portions, the outer (next to genital atrium) portion about 16–22 $\mu$ in diameter, lined with ciliated cells, the inner portion about 6–9 $\mu$ in diameter and without cilia (Fig. I, 2).

*Female reproductive system.* Ovary varying in shape and size with its development, and varying in position and shape with contraction of segment; the two lobes about equal in size; either lobe sometimes slightly smaller than the other. In a contracted mature segment 1·75 mm. wide by 0·6 mm. long, ovary 819 $\mu$ wide by 187 $\mu$ long; poral lobe 351 $\mu$ wide, aporal lobe 293 $\mu$ wide; vitellarium 462 $\mu$ wide by 82 $\mu$ long. Ovary and vitellarium disappearing in early gravid segments. Vagina conspicuous throughout its course even in *in toto* mounts, diameter variable; a dilatation frequently present near entrance of vagina into genital atrium, vagina sometimes measuring up to about 39 $\mu$ in diameter in this region, the remaining portion about 12–17 $\mu$ in diameter; vagina lined throughout with ciliated cells; uniting with seminal receptacle in region between lobes of ovary; shape and size of seminal receptacle dependent

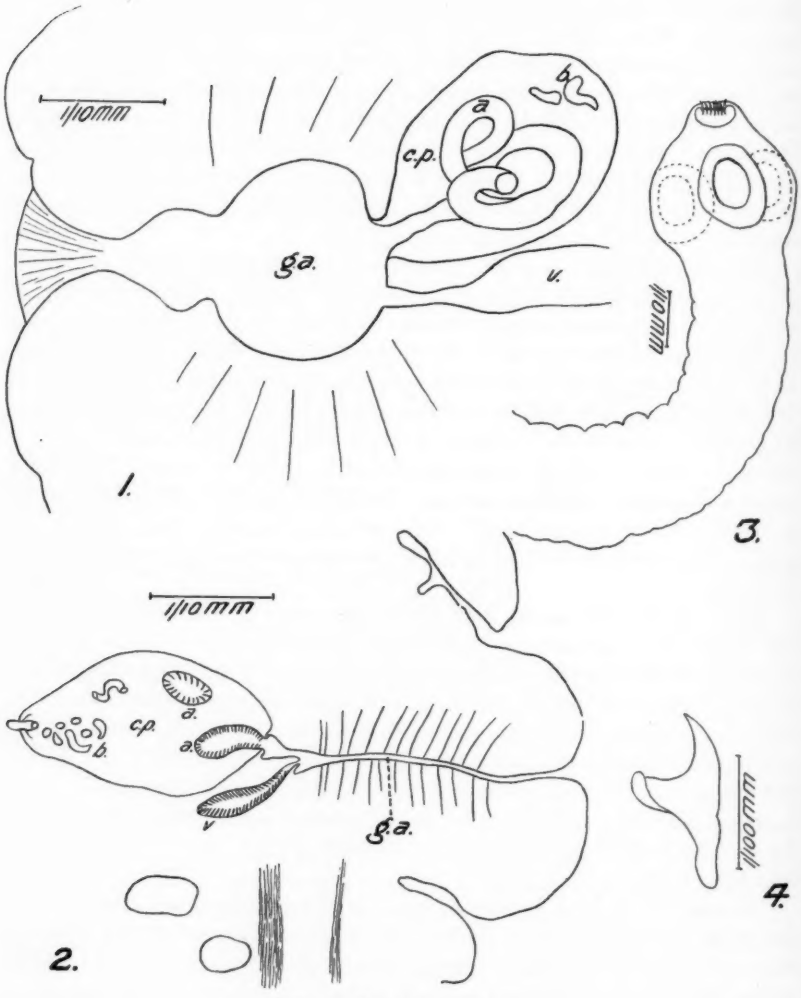


Fig. I. *Taenia tenuicollis.*

1. Region of genital atrium drawn from whole mount: *a*, ciliated portion of cirrus; *b*, non-ciliated portion of cirrus; *c.p.* cirrus pouch; *g.a.* genital atrium; *v.* vagina. 2. Region of genital atrium drawn from frontal section. Explanation of lettering as in 1. 3. Scolex. Hooks drawn free-hand. 4. Typical large hook of larva. Blade bent by pressure upon cover-glass.

upon its distension with spermatozoa; in both relaxed and contracted segments, vagina presenting several broad serpentine coils in region between lobes of ovary, the loops sometimes extending somewhat anterior to ovary lobes; vagina usually passing posterior to cirrus pouch but sometimes passing dorsal or ventral to it. Shell gland not demonstrable. Uterus at first a median tube, the first outpocketings appearing at its anterior end; lateral branches very variable in arrangement, usually impossible to count; in well-distended gravid segments, 10 to 19 lateral branches. Eggs (embryophores) 17–20 $\mu$ in diameter.

All specimens available for study showed contractions in nearly all segments; this gives far from "typical" conditions in all stages of development. If Cestodes can be said to show "typical" segments in the various stages of development, it is the belief of the writer that these can be demonstrated only in specimens which are relaxed.

*North American hosts.* Definitive: *Mustela vison*; intermediate: muskrat (*Ondatra zibethica* and *O. spatulata*). See "Life history".

*Location.* Small intestine of definitive host; liver of intermediate host.

*Distribution.* Canada, region near Kirkfield, Ontario.

*Life history.* In connection with the specimens from Canada, nothing based on life-history experiments is known, but the livers of several specimens of muskrats collected at different times in the region of Kirkfield, Ontario, show larval stages of a Cestode with hooks which agree approximately in size, shape, and number with those found in *Taenia tenuicollis*, therefore these larvae are tentatively identified as *Cysticercus talpae* Rudolphi, 1819. For further details concerning larvae, see accompanying description of *C. talpae.*

Some confusion apparently exists in regard to the correct name for this worm. Most of the synonyms listed have been queried for the reason that Rudolphi (1819) listed *Taenia putorii* and *T. mustelae vulgaris* as synonyms of *T. tenuicollis*; both these names had been listed by Rudolphi (1810) as "species dubiae", and for each of them he had listed several synonyms. Examination of the literature in order to trace any valid name which might have priority over *tenuicollis*, leads finally to *T. pusilla* and *T. serrata*, both published by Goeze (1782); *pusilla* receives priority on the basis of pagination. However, hosts for *T. pusilla* were rats and mice, while Rudolphi gave *Mustela vulgaris* as the host for *Taenia tenuicollis*; furthermore, the original description of *T. pusilla* gave no characters adequate for specific differentiation. In checking *T. serrata* one finds that on p. 350 (to which Rudolphi (1810) referred) Goeze recorded finding a Cestode from "einiger Iltisse und Mardern", and said it was much like the one he described as *T. serrata*, but he does not say they are identical; therefore, he cannot be said to have given the name *serrata* to the Cestode which is undoubtedly the only one described by him which can be considered similar to *T. tenuicollis* Rudolphi, 1819. Since all records earlier than those of Rudolphi (1819) yield no conclusive evidence for using any specific name other than *tenuicollis*, and since later records give adequate characterisation under the name *tenuicollis*, the present writer accepts *T. tenuicollis* Rudolphi, 1819 as the

name for the species herein redescribed. The original description of *T. tenuicollis* cannot be said to provide conclusive specific characters, but Dujardin's description of a Cestode also from *Mustela vulgaris* (type host for *Taenia tenuicollis*) adds to the original description the characters of the small hooks and the very prominent genital atrium which are two of the best characters for separating the species considered by the present writer to be *T. tenuicollis* from other members of the genus *Taenia*. Furthermore, re-examination of Rudolphi's specimens by Thienemann (1906) resulted in Thienemann accepting Rudolphi's specimens as identical with those described by Dujardin (1845); this fact, combined with the similarity in the size, shape, and number of the hooks and the prominence and location of the genital papillae in the forms described by both Dujardin and Thienemann, leaves no doubt that Dujardin and Rudolphi dealt with the same species.

*Taenia tenuicollis* Rudolphi, 1819 is frequently confused with *T. intermedia* Rudolphi, 1810, but Rudolphi described *T. intermedia* as having a double row of powerful little hooks ("corona uncinulorum validorum duplici cinctum") in contrast to the absence of hooks ("rostello inermi") in *T. tenuicollis*. The writer assumes that hooks which Rudolphi would have described as powerful were relatively large, while hooks of the size (15–20 $\mu$) of those of *T. tenuicollis* may have escaped his notice or have been lost; re-examination of Rudolphi's material (*T. tenuicollis*) by Thienemann (1906) showed that no hooks were present at the time of his examination. Thienemann stated that the drawings he gave as hooks of *T. tenuicollis* were made from specimens of the larva, *Cysticercus innominatus hypudaei* (= *C. talpae*). Dujardin (1845) gave drawings (Fig. II, 9 and 10) of hooks of *Taenia tenuicollis* and stated his conviction that these specimens described by him as having 52 hooks 20 $\mu$ long were the same species as those described by Rudolphi as being without hooks. Dujardin (1845) also described the hooks of *T. intermedia* as being large ("forts"), and added that *T. intermedia* may be distinguished from *T. serrata* of the dog by the elongated head and the longer posterior segments of the former. Dujardin's comparison of *T. intermedia* with *T. serrata* (presumably *T. pisiformis* in this case since Dujardin wrote "*Taenia serrata* du chien") supports the viewpoint that the hooks of *T. intermedia* are much larger than those of *T. tenuicollis* and are approximately the size of those of *T. pisiformis*. Küchenmeister (1855) gave a drawing (Fig. II, 12) of the hooks of *T. intermedia* drawn to the same scale as those of *T. pisiformis*; from this drawing, estimating the large hooks of *T. pisiformis* to be about 250 $\mu$ long, those of *T. intermedia* prove to be about 180 $\mu$ long. Leuckart (1856) gave excellent drawings of the hooks of *T. intermedia* (Fig. II, 13*a*, *b*), *T. taeniaeformis*, and *T. tenuicollis* (Fig. II, 11), and since the scale of magnification is given, the length of those of *T. intermedia* may be estimated and proves to be about 180 $\mu$. Thienemann (1906) re-examined Rudolphi's specimens of *T. intermedia* and gave a drawing of a hook which, judging from the shape, must be considered a large one; he described the large hooks as being 145 $\mu$ long; this is conclusive evidence of the size and

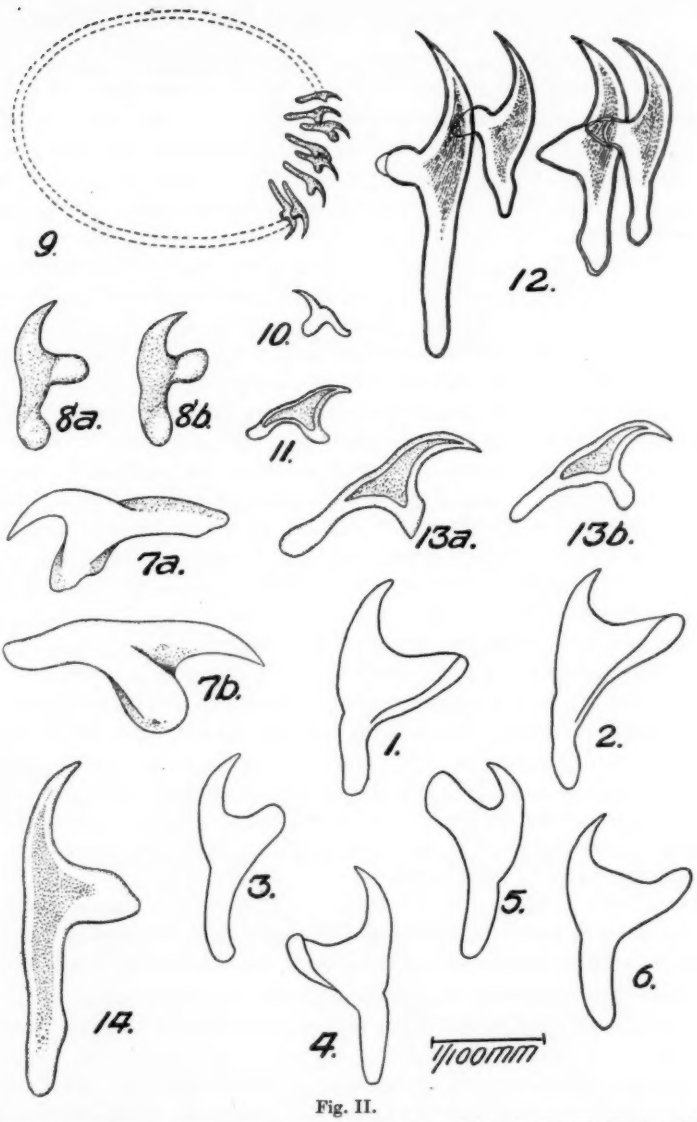


Fig. II.

1–6. Hooks of *Cysticercus talpae*. Drawn to show variation. Original. 7*a*, 7*b*. Hooks of *Taenia tenuicollis* drawn from larva. ×1130. After Thienemann (1906). 8*a*, 8*b*. Hooks of *Taenia tenuicollis*. Enlarged. After von Linstow (1884). 9, 10. Hooks of *Taenia tenuicollis*. 9. Entire circlet. ×430. After Dujardin (1845). 10. Isolated hook. ×450. After Dujardin (1845). 11. Hook of *Taenia tenuicollis*. ×700. After Leuckart (1856). 12. Hooks of *Taenia pisiformis* (on the left); hooks of *T. intermedia* (on the right). Drawn to show relative size. Enlarged. Adapted from Küchenmeister (1855). 13*a*, 13*b*. Hooks of *Taenia intermedia*. ×175. After Leuckart (1856). 14. Hook (presumably the larger one) of *Taenia intermedia*. ×420. After Thienemann (1906).

shape of the hooks of *T. intermedia*. However, Thienemann said that the measurements of the hooks given by him agreed with those given by Leuckart (1856), but the present writer does not find this to be the case. Thienemann said of the large hooks: "Entfernung von der Sichelspitze nach dem Ende des Wurzelfortsatzes 0·145 (0·14) mm."; in a footnote he said the figures in parenthesis were those given by Leuckart, but Leuckart said, "die Lange des Wurzelfortsatzes = 0·14 ist"; since all Leuckart's measurements are in millimetres, it must then be considered that he said the length of the *handle* or *root* is 140 $\mu$, while Thienemann said the length of the *entire hook* is 145 $\mu$. Fuhrmann (1926) described the hooks of *T. intermedia* as 140 $\mu$ long. The present writer considers the length of the large hooks of *T. intermedia* to be 145 $\mu$, but sufficient evidence is cited to show that although there is sufficient variation in the size and shape of the hooks of forms reported as *T. intermedia* to suggest that more than one species has been reported under that specific name, there is, at least, agreement among the various authors in regard to the hooks of *T. intermedia* being large and quite unlike those of *T. tenuicollis*. Baer (1932) found in *Arctogale erminea* (= *Mustela erminea*) and *A. nivalis* (= *Mustela nivalis*) fragmentary specimens which he considered to be *Taenia tenuicollis* because they agreed with the specimens described by Thienemann. Since his specimens had no hooks, Baer thought it possible that *T. tenuicollis* is a species without hooks, and that the hooks drawn by Leuckart and Dujardin were hooks of *T. intermedia*; this viewpoint is not acceptable when Dujardin's comments on *T. intermedia* are added to his description of *T. tenuicollis*, or when one accepts the measurements given by Thienemann for the hooks found on Rudolphi's specimens of *T. intermedia*. Furthermore, Baer described his specimens as having a very large genital atrium with very muscular walls, and this is a conspicuous character of the form with small hooks which the present writer considers *T. tenuicollis*. It is the writer's opinion that Baer's specimens were undoubtedly correctly determined.

Baer (1925) considered *T. intermedia* a synonym of *T. taeniaeformis*, and this would create further confusion were it not for such evidence as Leuckart's (1856) drawings, which clearly differentiate *T. intermedia* and *T. taeniaeformis*, and the evidence presented by Thienemann after re-examination of Rudolphi's material. It may be that one of these names involved in this synonymy is a misprint.

Cameron (1933) reported Cestodes from *Mustela erminea* and *M. nivalis* which he considered to be *Taenia intermedia*. These he described as having 36–40 small hooks 20 $\mu$ long, large ventral excretory vessels, a conspicuous genital atrium, the vagina a wide undulating tube, and 16–20 uterine branches. All these characters agree with the form which the present writer considers *T. tenuicollis*, and the figures given by Cameron give further evidence that the specimens are that species. Cameron (1933) also reported *T. tenuicollis* as being differentiated from *T. intermedia* by the former lacking hooks and having a less conspicuous neck, narrow excretory vessels, and a rather small number of

uterine branches. It is the writer's opinion that these specimens which he thus described cannot be *T. tenuicollis.*

Linstow (1884) gave a good figure (Fig. II, 8*a*, *b*) of the hooks of *T. tenuicollis* and described the larger hooks as being 24 $\mu$ long and the smaller ones as 21 $\mu$ long. Braun (1896) pointed out that Leuckart (1863) was in error when he considered *Cysticercus talpae* the larvae of *Taenia intermedia* since Leuckart noted expressly that the hooks of *Cysticercus talpae* were very small and, according to Braun, could not therefore be considered hooks of *Taenia intermedia*, because the hooks of the latter are about the size of those of *T. solium.* Braun also suggested that Rudolphi had overlooked the hooks of *T. tenuicollis.* Sufficient evidence has been cited to show that *T. intermedia* cannot be confused with *T. tenuicollis* or with *T. taeniaeformis* and that there is an adult Cestode found in Mustelidae which has hooks very much smaller than those of other members of the genus *Taenia.*

Meggitt (1924) included "*T. tenuicollis* Rudolphi" as a synonym for *T. hydatigena*, but the latter is a valid species and adequate evidence is given in the foregoing description to show that *T. tenuicollis* cannot be considered a synonym of a form with large hooks such as are found in *T. hydatigena.*

Cameron (1933) and Baer (1932) considered *T. brevicollis* a synonym of *T. tenuicollis*, but Rudolphi (1819) stated that the former was like *T. intermedia*, from which he separated it on account of the branched ovary in *T. brevicollis*; the ovary of *T. intermedia* he described as unbranched. Sufficient evidence is cited by the present writer to establish the validity of *T. intermedia*, and Thienemann (1906) re-examined Rudolphi's specimens of *T. brevicollis* and on comparison with *T. intermedia* and *T. tenuicollis* he concluded that, even in the absence of hooks from *T. brevicollis*, it was distinct from *T. tenuicollis* and *T. intermedia.* It is, therefore, the opinion of the writer that *T. brevicollis* should stand as *species inquirenda* rather than as a synonym of either *T. intermedia* or *T. tenuicollis.*

### **Cysticercus talpae** Rudolphi, 1819

SYNONYM: *Cysticercus innominatus hypudaei* Leuckart, 1857.

Livers of three specimens of *Ondatra zibethica* were collected by Dr R. G. Law of the Experimental Fur Farm in the vicinity of Kirkfield, Ontario. These show heavy infestation with cysts containing larval tapeworms.

*Description.* Cysts whitish in colour, usually roughly ovoid in shape; size variable, from about 2 mm. long by 1 mm. wide up to about 8 mm. long by 4 mm. wide. Sometimes only one scolex in each cyst, sometimes two or three scoleces, sometimes many. Scolex about 225 $\mu$ in diameter at suckers; rostellum about 70 $\mu$ in diameter; suckers about 110 $\mu$ in diameter. Two characters useful for specific differentiation are present: (1) Rostellar hooks 42–60 in number, in two rows, hooks of both rows similar in shape, guard prominent (Fig. I, 4) and bifid in both rows of hooks; hooks of anterior row 16–20 $\mu$ long; hooks of posterior row about 16–18 $\mu$ long. (2) Body wall of larvae showing irregular

transverse striations about 6–12 $\mu$ wide, these increasing in prominence in region of scolex; striations with saw-like edges; each tooth-like protuberance about 3–6 $\mu$ wide; width of striations in normal position difficult to determine since flattening of larva is necessary in order to determine details of striations. Of the three collections of specimens from livers, only two showed these striking transverse striations, and in these two no calcareous corpuscles were present; in the third liver, which had evidently been very differently treated in fixation, since it had become ivory coloured whereas the other two remained dark red, the specimens showed no striations, but the tissues of these larvae were so disintegrated that even the suckers were only rarely visible, and the hooks, which in the other two specimens could not be separated to secure a lateral view of them, were readily separated by pressure upon the cover-glass. These disintegrating larvae showed numerous calcareous corpuscles, ovoid or round in shape, from 5–22 $\mu$ in greater diameter; no calcareous corpuscles demonstrable in specimens from the two red livers. It is assumed that difference in fixation of the livers accounts for these differences in the larvae, since all hooks were similar in size and shape.

The hooks of these larvae agree approximately in size and number with those of *Taenia tenuicollis*, and, furthermore, these larvae are found in the same locality in which *T. tenuicollis* is found; therefore, the larvae are tentatively determined as *Cysticercus talpae*. The slight difference in the length of the hooks of the larva and the hooks of the adult may be accounted for by the fact that many crowns of hooks of the larvae were examined and measurements made on hooks which had been flattened to secure a lateral view, thus including the slight variations in length, while in the adult form it was impossible to secure a lateral view and accurate measurement of hooks could not be made. Experimental evidence is required to settle the question conclusively, and life-history experiments are being carried out for the purpose of securing such evidence. If these experiments yield conclusive results, report of it will be made elsewhere. The number of scoleces found in each bladder varies; in some livers the single scolex in a bladder is common, while in another liver the usual form may be a bladder containing many scoleces, but both forms occurred in every liver examined by the writer. The occurrence of these two conditions in the same species of Cestode is of interest, confirming Villot's (1883) observation that many scoleces may be found in the bladder of *Cysticercus talpae*. According to Villot, proliferation has also been observed in the cysticerci of other species of the genus *Taenia*, namely, *Cysticercus cellulosae*, *C. tenuicollis*, *C. pisiformis*, *C. longicollis*, and *C. fasciolaris*. These forms establish a transition between the cysticercus with one scolex and the coenurus with many scoleces. Of the few details which have been published concerning the European specimens, the size and number of hooks and the facts that the bladders are of the "proliferating" type, and, according to Braun (1896), the hooks are well developed on the 50th day but are lost by the 123rd day, are the chief ones brought out by earlier authors. The variation in the size of the completely developed hooks

is only slight, if any exists; the apparent variation is probably due to the difficulty of securing a perfectly flat hook for measurement; they are invariably turned so that either the tip or the handle is much out of focus, and pressure upon the cover-glass adequate to adjust this rarely does more than bend the guard somewhat. Since hooks have been observed on the adult specimens by Dujardin and the present writer, it is difficult to explain Braun's statement that hooks are usually lost by the 123rd day. The probability that the larvae described by the present writer as *Cysticercus talpae* may not be the larval form of the adult specimens collected from the mink in the same locality is only slight, but since the lateral view of the hooks of the adult Cestodes from Canada has not been observed, there is a possibility that the guard may be unlike that of the larval forms from the muskrat in that region; in an *en face* view of the rostellum the hooks of *Taenia tenuicollis* are similar to those of the larva described in this paper as *Cysticercus talpae*.

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# THE SECOND ECDYSIS OF INFECTIVE NEMATODE LARVAE

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(With 6 Figures in the Text)

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## I. Material and methods

The infective larvae used were those of *Haemonchus contortus*, *Ostertagia circumcincta* and species of *Trichostrongylus*. With the exception of a few cultivated on partially sterilised sheep's faeces in the usual way, they were all reared by the method described by McCoy (1929, 1929*a*) and the writer (1933) on pure cultures of intestinal bacteria, the organism used being a strain of *B. coli* isolated from the intestines of the nematode larvae themselves.

The writer first used hypochlorite solutions for the artificial production of the second ecdysis and for the sterilisation of the exsheathed larvae as a result of observations, made in 1930, on the effect of Liquor Sodae Chlorinatae (B.P.) on infective larvae of *H. contortus*. Various solutions containing hypochlorite were tried, but were discarded because of their instability. The solution sold under the name of "Milton" was finally adopted, because its effects were the same although the same bottle of it was used for longer than a year. Undiluted Milton was found to be too strong. At 15–20° C. the larvae were active for only a few seconds in it and their sheaths were no longer visible on them; later the larvae themselves were dissolved. Ecdysis occurred in 10 sec. at 15–20° C. in Milton 1 part, distilled water 4 parts, the larvae being violently active in this. In Milton 1 part, distilled water 9 parts, ecdysis occurred in 4 min. as a rule and the cast sheaths were dissolved in 23 min. The exsheathed larvae lived 3–4 days in this dilution, and one larva lived 20 days in it, in sealed hanging drops at 38° C. Some larvae, however, were dissolved in these sealed and incubated drops, probably because the hypochlorite content of different drops varied.

In Milton 1 part, distilled water 19 parts, all the larvae exsheathed in 20–25 min. at 15–20° C. The cast sheaths were not so quickly dissolved, but the exsheathed larvae were healthy, and both the larvae and the dish containing

them were sterilised. This dilution was therefore adopted for standard use. Still weaker dilutions of Milton caused ecdyses after a longer time had elapsed, but these did not sterilise the larvae and there seemed to be no advantage in using them.

The usual technique adopted was to transfer the larvae from a culture with a modified Rees (1930) pipette to sterile water in a solid watch-glass to allow them to wash themselves clean of some of the bacteria transferred with them; they were then transferred with a fresh Rees pipette to the Milton solution in another watch-glass or in the kind of well-slide used for tissue culture. The ecdysis could then be observed 25 min. later. Sometimes single larvae were removed to hanging drops sealed with paraffin for observation of the ecdysis by higher magnifications. If sterilised exsheathed larvae were required, they were removed from the Milton solution with a sterile Rees pipette to sterile water, and, after they had washed themselves free of Milton, they were transferred to various media, either in dishes or in sterile hanging drops of the type used for tissue culture. The attempts thus made to cultivate the larvae further will be described in another paper.

### II. Ecdyses in 1 in 20 dilutions of Milton with water

Ecdyses occur so rapidly in this solution that the details observable during the slower ecdyses in mixtures of HCl and NaOH, described below, are not visible; nor was any detail seen which constantly gave warning that ecdysis was about to occur. Larvae often cease to swim about just before exsheathing and then they writhe and coil very actively without leaving the field of the microscope; but this behaviour is normal and is not a constant prelude to ecdysis. The first detail seen is the sudden bursting of the anterior tip of the sheath and the sudden, but slow, expulsion of the larva through it, until it is half-way out of the sheath or less. The impression given is that considerable pressure exists inside the sheath when it bursts and the experiments described below show that such an increased internal pressure does, in fact, exist.

The remarkable and abnormal feature of these ecdyses in Milton is that the anterior end of the sheath is never thrown off as a conical cap in the manner characteristic of normal ecdyses. The reason for this can best be explained after the ecdyses in mixtures of HCl and NaOH have been described below. The ecdyses in Milton are, nevertheless, just as successful as normal ones, releasing perfectly normal and healthy larvae.

The larval movements usually cease while the larva is being propelled out of the sheath for a half or so of its length. The larva then becomes active again and extricates itself from the sheath by its own movements. Provided that the larva is normally active, it has usually completely emerged a few seconds after the bursting of the tip of the sheath. In the stronger dilutions of Milton its emergence may seem almost instantaneous, no distinction being observable between the initial passive propulsion of the larva part of the way out and its completion of the ecdysis by its own movements. During this latter phase the

tail of the larva can be seen moving along the interior of the sheath until it reaches the hole at the anterior end, where it may sometimes stick, perhaps because the tail is slightly curved or because the frayed margins of the aperture have collapsed inwards and partly occlude the outlet. In such instances the larva, by violent lashing movements, frees the tail suddenly by giving the sheath, so to speak, a violent kick. Occasionally, before they do this, larvae swim about for a while dragging their sheaths after them. The empty sheaths usually collapse in segments as the larvae leave them, their sides falling in at intervals to occlude the lumen.

Healthy larvae have no difficulty in leaving the sheaths. A delay of a few seconds may occur after the initial passive expulsion, especially when this does not carry the larva very far out. The behaviour of the larva then suggests that only a small aperture was made when the sheath burst at its tip, wide enough to let out the narrower oral end of the larva, but not wide enough to let out the widest part of the larva at the base of the oesophagus. Usually this difficulty is easily and suddenly overcome by the larval movements. Sometimes, however, especially if the larvae are not as active as usual, these movements fail to extricate the larva so that it dies partly out of its sheath; or its emergence may be very much delayed, as in the instance of one such larva which required 143 minutes to extricate itself. Alternatively the internal pressure released when the larva is first passively expelled may reaccumulate against the resistance of the larva impacted in the opening at the anterior tip of the sheath, and when it reaches sufficient force, may expel the larva passively, or may stimulate it to renewed movements, if these have ceased, so that it emerges successfully. In either instance, the final expulsion of the larva must exert considerable force on the margins of the opening of the tip of the sheath and this may explain why the edges of this opening are often frayed out, as if they had been torn. Similar fraying may, however, also be seen when larvae emerge in the normal manner by the detachment of a cap, or during the ecdyses of larvae whose sheaths have been considerably distended by reagents.

All these details point to the existence of considerable internal pressure, which combines with specific movements of the larva, described below, to effect the ecdysis. Only rarely do ecdyses occur by the larval movements alone, or by the internal pressure alone expelling the larvae completely without any movements of their own. This pressure is chiefly due to the osmotic factors presently to be described, but may be partly due also to accumulation of excretory products of the larva.

Very few variations from the process just described have been observed in hypochlorite solutions. In a few instances larvae emerged by a rent at the side of the oesophageal region, in the manner described as characteristic of the third ecdysis by Veglia (1915) and the writer (1933). In only one instance the whole sheath split suddenly down two-thirds of its length and the larva wriggled out by a single movement. It is possible that in this instance the sheath gave way along one of its lateral lines owing to the factors described below.

### III. EFFECTS ON THE LARVAE REGARDED AS BEING THOSE OF CHLORINE

There is not space to describe here in detail the large number of experiments done in an attempt to explain the action of Milton and other hypochlorite solutions. These experiments showed that ecdyses cannot be caused by chlorine, oxygen, carbonic acid, NaCl, nor NaOH alone. An attempt to differentiate between the action on the sheaths of chlorine and hypochlorite revealed interesting effects which were regarded as those of chlorine. These were observed when larvae were put into chlorine water not strong enough to kill them, or were exposed to the vapour of this; and also when larvae were put into hypochlorite solutions to which excess of acid had been added to decompose the hypochlorite into chlorine and water, or were exposed to the vapour of these, which was presumably that of chlorine. Some of the hypochlorite solutions were thus acidified with HCl, but the solution most used was Milton acidified with acetic acid in the proportion of 0·25 c.c. of 10 per cent. glacial acetic acid in distilled water and 5 c.c. of a 1 in 20 dilution of Milton in distilled water. The addition of this amount of acid prevented the ecdyses which normally occur in this dilution of Milton, acetic acid alone having no power to cause them. The mixture must be freshly made. If it is left uncovered, it does not produce the effects here described, a fact which further suggests that these effects are those of chlorine.

The effects observed were:

(1) That no ecdyses occurred in these solutions alone.

(2) That the permeability of the sheaths was so altered that they became swollen out away from the larvae and obviously distended by the accumulation of fluid within them.

(3) That frequently the outer layer of the sheaths was blistered out, as if the sheath had split into two layers, which perhaps correspond to the two layers of the cuticle of adult Ascarids described by Mueller (1928–9) as having different chemical compositions.

(4) That the distended sheath, whether it was thus blistered or not, looked to the eye much thinner. That this was not merely the optical effect due to the stretching of it by distension is suggested by the fact that the sheaths were often so much thinned that they appeared to the eye like very diaphanous membranes which later collapsed in folds on to the larvae as the osmotic forces adjusted themselves to equilibrium, and these crumpled sheaths then still retained their extremely diaphanous appearance, *i.e.* even after the internal tension had relaxed.

(5) That the cap at the anterior end, which is detached at the normal ecdysis to liberate the larva, was not distended like the rest of the sheath, or was only slightly distended when the distension of the rest of the sheath was very marked. It was only rarely blistered and never appeared to be thinned. It therefore appeared to be less elastic and less readily affected by the chlorine vapour. These facts suggest that the chemical composition of the cap may be

slightly different from that of the rest of the sheath. Their important bearing on the explanation of the ecdysis is further discussed below.

(6) That the degree of distension of the larval sheaths by this treatment could be controlled by altering the salt content of the medium in which the larvae were. If they were exposed to the vapour of chlorine water or to that of acetic-Milton while they were in hanging drops of distilled water, or if they were transferred to distilled water after they had been in chlorine water or acetic-Milton, the distension was greater than when weak solutions of $Na_2HPO_4$ or sodium borate were used instead of distilled water. This distension of the larvae, however, gradually decreased the longer the larvae remained either in distilled water or in these solutions. Further, the initial distension was less when the larvae were put, after treatment with acetic-Milton, into stronger solutions of these salts. Thus when they were put into a 0·5 per cent. solution of $Na_2HPO_4$, marked distension followed and gradually decreased the longer the larvae were in it, until the sheath returned practically to normal dimensions. In 10 per cent. $Na_2HPO_4$ distension still occurred: in a 13·3 per cent. solution very little occurred; in a 20 per cent. solution there was first distension and then shrinkage of the larvae till they occupied only half the sheath and were crumpled up and dead. A solution at or near 13 per cent. seemed, therefore, to be isotonic to larvae whose sheaths had been altered by acetic-Milton.

In a similar way it was found that a solution of sodium borate at or near 10 per cent. was isotonic to larvae similarly treated.

Larvae transferred to physiological saline after treatment with acetic-Milton showed marked distension, so that this was then hypotonic to them, although no such change appeared when larvae not treated with acetic-Milton were put into physiological saline, in which they lived very well. A solution of NaCl at or near 5 per cent. was isotonic to the larvae after treatment with acetic-Milton. Any stronger solution of NaCl caused shrinkage of the larvae in their distended sheaths.

(7) Ecdysis sometimes occurred in these solutions of $Na_2HPO_4$, or sodium borate, after larvae had been treated with acetic-Milton, but never in the chlorine water or acetic-Milton alone. These ecdyses were, however, never effected by the detachment of a typical cap, nor by the dissolution of the sheaths. They were due to the activity of the larvae, which broke their way out of the thinned and distended sheaths. The sheaths were never dissolved in these solutions.

(8) The sheaths were only dissolved when the larvae, after treatment with chlorine water or acetic-Milton or the vapour of these, were transferred to weak solutions of NaOH (0·4 per cent. or stronger). This indicated that the chemical composition of the sheaths had been so altered by the chlorine that they had become soluble in NaOH. This chemical change in the sheaths was perhaps also the cause of the alteration in their permeability, as a result of which they became distended.

Ecdysis could, therefore, be induced by treating the larvae with chlorine

water or acetic-Milton not strong enough to kill them and then transferring them to weak NaOH, when the larvae were set free, not through a hole at the anterior tip of the sheath, as occurs in diluted Milton, but by the dissolution of their chemically altered sheaths by the NaOH, or alternatively by the weakening of the sheaths so that the larvae could break out.

That this is also the explanation of the action of Milton there can be little doubt. A 1 in 20 dilution of Milton contains more than enough chlorine and NaOH to produce the effects just described; it also contains about as much NaCl as physiological saline and is therefore hypotonic to the larvae. The reason that no obvious distension of the sheath occurs in it is that the chlorine alters the sheath so rapidly that it very quickly becomes soluble in the NaOH present, so that the anterior tip, its weakest part, gives way as a result of both this solvent action of the NaOH and of the increased internal pressure due to the alteration in its permeability, before any obvious distension can occur. In stronger dilutions of Milton the same thing occurs, but the sheath is dissolved off either before the larva has left it or while it is doing so; even in 1 in 20 dilutions the sheaths are always dissolved after the larvae have left them.

This rapidity of the action of the chlorine and the NaOH in the Milton also indicates why no cap is detached when ecdyses occur in hypochlorite solutions. Ecdyses are described below in weak mixtures of HCl and NaOH, in which a cap is detached as it is when ecdyses occur in nature; but when these ecdyses occur in mixtures of HCl and NaOH, a period of time must elapse during which the chemical composition of the sheath is being altered, with the result that the internal pressure is increased and the sheath is rendered soluble in NaOH; and in addition specific movements of the larva must come into play to determine the point at which the undistended and less elastic oral end of the sheath which forms the cap is to be detached. If, for any reason, these additional factors do not take effect, no cap is thrown off. The distended sheath simply bursts at its weakest point; and this is what happens when ecdyses occur in hypochlorite solutions.

The tip of the sheath, where it bursts when ecdyses occur in Milton, is likely to be its weakest point because the mouth of the second larva, before the sheath separated from it, lay at this point, where its remains can usually be seen, together with an indentation where the mouth of the second larva was; and this is also the last portion of the sheath to separate off when the larva becomes a mature infective larva. In bacterial cultures larvae can be seen with all parts of their sheaths separated from the third larva inside, excepting only the portion at the margins of the mouth, which is still ingesting bacteria. Until the sheath also separates here the third larva is not shut off from the outer world and is therefore not a mature infective larva. For both these reasons the sheath at this point may be less able to resist the action of chlorine followed by that of NaOH.

The conclusion thus tentatively drawn, that the ecdyses so far observed were due to the action of chlorine followed by that of NaOH, seemed to throw

little light upon ecdyses that occur in nature, unless it were legitimate to suppose that these also are due to an alteration in the chemical composition of the sheaths by such factors as alternate drying and wetting, exposure to heat and cold, the acidity of cultures and so on, as a result of which the permeability of the sheaths is altered and they become soluble in alkali. An hypothesis of this kind would explain the shrinkage of larvae inside their sheaths in old cultures and similar abnormalities of their relations to their sheaths; it would also explain why ecdyses occurred when Veglia (1915) put larvae into the mouths of sheep, where they would encounter the alkaline saliva. Ecdyses might similarly occur in the duodenum or even in the alkaline rumen contents [*p*H 7 (Mangold, 1929)]. If the sheaths of larvae had not been altered before they entered their host, exposure of them first to the HCl in the gastric juice and later to the alkaline duodenal contents might be equivalent to exposure of them to the factors described above; and this might be the explanation of ecdyses that occur normally in the body of the host. Before this possibility could be entertained, however, it was necessary to show that ecdyses would occur in weak mixtures of salts in which chlorine and OH ions were present, or in solutions containing Cl ions followed by those containing OH ions. Larvae were, therefore, put into hanging drops composed of mixtures of solutions of HCl and NaOH of strengths at any rate not greater than those at which these compounds occur in the stomach or intestine, and also into weak HCl, after which they were washed in water and transferred to weak NaOH.

### IV. Ecdyses in solutions containing HCl or NaOH or both

#### 1. *In HCl alone*

Veglia (1915) saw only a few ecdyses of *Haemonchus contortus* larvae in 1 and 5 per cent. solutions of HCl; 5 per cent. HCl killed his larvae in 24 hours; 1 per cent. HCl killed 60 per cent. of them in 3 days. Mönnig (1926–7) found that "weak HCl" caused larvae of *Trichostrongylus* to exsheath; it made them very active and caused them to congregate in clusters, as the writer also observed. The writer, however, could never obtain ecdyses in any solution of HCl alone. It is possible that Mönnig's "weak HCl" was made up with water containing enough OH ions to complete the ecdyses in the manner described below.

#### 2. *In NaOH alone*

Goodey (1922) found that the sheaths and larvae of *Graphidium strigosum* and *Trichostrongylus retortaeformis* of the rabbit were dissolved away overnight in 5 per cent. NaOH at 37° C. and in 1¼ hours in 15 per cent. NaOH. The writer found that antiformin, composed of equal parts of Liquor Sodae Chlorinatae (B.P.) and a 15 per cent. solution of NaOH, had a similar effect.

In watery NaOH solutions the writer found that ecdysis never occurred regularly. Some "older" larvae from faecal cultures exsheathed in 5 per cent. NaOH at 31° C. and next day both the sheaths and the larvae had been dis-

solved. Other larvae, which had been 42 days in a *B. coli* culture since they had become mature, were put into 0·5, 1 and 2 per cent. solutions of NaOH in sealed hanging drops at 38° C. When they were examined 2 hours later they were all dead, and were so transparent that they were difficult to see. It could be seen, however, that their sheaths were distended. In the 0·5 per cent. solution, although their sheaths remained just visible by careful lighting, the larvae and their contents were reduced to a cluster of granules only just visible; they retained, however, the general form of the larva, which suggested that the skin of the larva, as well as the sheath distended away from it, was still intact. In the stronger solutions the same changes occurred, but more rapidly, and the larvae were reduced in the 2 per cent. solutions to mere vestiges represented by scarcely visible granules, although their sheaths remained just visible. These larvae did not undergo ecdysis because the solution rendered them inactive at once, so that the necessary action of their own movements, described below, could not come into play; and the stronger solutions, at any rate, killed them almost at once.

In these NaOH solutions the sheaths were also sometimes blistered, as they are in chlorine water. It was evident that, before these larvae were put into these NaOH solutions, changes had occurred in their sheaths analogous to those described above as the effect of chlorine water and acidified Milton, because their sheaths had been rendered soluble, or partially soluble, in NaOH. Experiments done in 1930 with larvae from faecal cultures had already given the impression that ecdyses occurred more readily in alkaline digestive media, such as artificial pancreatic juice and extracts of duodenal mucosa, than in acid gastric juice or extracts of the abomasal mucosa. It is well known that old faecal cultures are usually acid, so that the more frequent ecdyses observed in these alkaline media may have been also due to alterations in the sheaths by acids in the cultures which rendered them partially or wholly soluble in alkali.

### 3. *In $Na_2CO_3$*

In 1 per cent. solutions of $Na_2CO_3$, which dissociate into NaOH, $CO_2$ and $H_2O$, larvae taken from a *B. coli* culture 42 days after they had become mature in it, were all dead after 8 days at 38° C., but some had exsheathed by a typical cap, and their empty sheaths had been dissolved. Experiment had already shown that neither $H_2CO_3$ nor $CO_2$ can alone produce these changes. The general effect of $Na_2CO_3$ solutions was like that of the mixtures of HCl and NaOH described below, but varied much more, and sometimes no change was produced. This variation probably depended on how far the sheaths had already been changed in the cultures from which the larvae were taken. The sheaths of those which showed no change in the NaOH had been, presumably, not altered at all.

### 4. *In mixtures of NaOH and HCl*

All the larvae put into these mixtures were studied throughout at 38° C., in hanging drops sealed with paraffin-wax, like those used for tissue culture. The other experiments described above were done at room temperature

(18–22° C.). These facts should be taken into account in any interpretation of the appearances produced, especially perhaps when this involves the theory of ionisation. In putting forward the interpretation given below, the writer intends to do no more than indicate possibilities which may seem worth further investigation by workers competent to deal with biophysical problems in organisms which are not, as a rule, larger than half a millimetre in length.

The mixture of HCl and NaOH found to be most suitable for causing the ecdyses now to be described was made up of 0·5 c.c. of a 0·5 per cent. solution of NaOH and 1 c.c. of a 0·5 per cent. solution of concentrated HCl, the *p*H of this, as determined by the B.D.H. capillator, being 9–10.

Ecdyses in this mixture required a comparatively long time, about 8 days at 38° C. being the average time needed by larvae in hanging drops sealed with paraffin. This may be a delayed process, but no evidence was obtained that it was in any way abnormal.

All these ecdyses, unlike those in Milton described above, occurred by the detachment of a typical cap from the anterior end of the sheath; but the detachment of this cap was apparently effected by a process similar to that governing the ecdyses in Milton, namely, an alteration in the permeability of the sheath, which resulted in an increased internal pressure with distension of the sheath.

The first indication that an ecdysis might occur was the appearance of a slight swelling or fold of the sheath at the point where the base of the conical cap is subsequently detached (Fig. 1). This slowly became more pronounced and gave the impression that the pressure inside the sheath had increased. In many instances the sheath looked thinner than usual before this swelling or fold appeared, but this appearance of thinning is seen in all solutions containing NaOH.

Larvae which showed this swelling were often slightly retracted in their sheaths and this increased the impression of high internal pressure and distension. Practically all larvae, however, taken from cultures a few days after they reach maturity show some degree of retraction. It is probably due to alteration of the permeability of the sheaths and therefore occurs only when larvae are in solutions hypertonic to them, such as, for example, older cultures from which evaporation has occurred.

A little later these swellings of the sheath had the appearance shown in Figs. 3 and 4. At the region of the swelling a separation of the sheath into two layers had occurred. The outer one was swollen out all round this region of the larva and to an equal degree all round, while the inner layer was either broken as Fig. 4 shows, or as yet unbroken, or broken only on one side of the particular optical section of the larva being examined (Fig. 3).

These two layers of the sheath cannot, of course, be seen in normal larvae; but they are also evident when the sheath is blistered by chlorine and acidified Milton or the vapour of these, which do not cause ecdysis. Mueller (1928–9) has described two layers in the cuticle of adult Ascarids, which he regards as

having different chemical compositions, and it is possible that the sheath of the infective larva of all nematodes consists of two similar layers, because it is the old skin of the second larva, and we have as yet no reason to suppose that the skin of the larva differs from that of the adult, except in thickness.

In some larvae at or about this stage, the appearance shown in Fig. 6 was seen, especially when the larvae were much retracted, and were sluggish enough to be seen with higher magnifications. This figure shows the broken ends of the inner layer of the sheath. Their wavy outline and the wide space

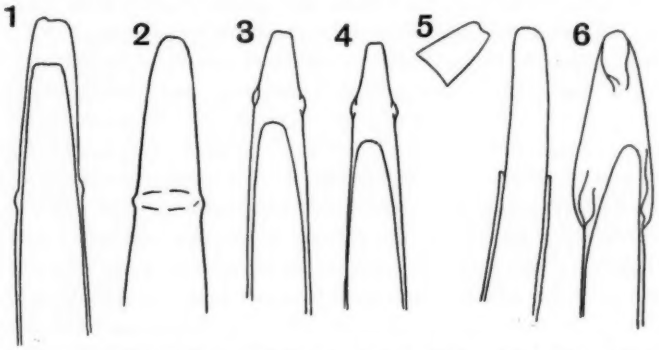


Fig. 1. Shows the swelling at the base of the cap which is the first visible change in the sheath during ecdyses in mixtures of HCl and NaOH. ×300.

Fig. 2. Indicates by a dotted line the refractile ring which appears when the inner layer of the sheath is ruptured as in Figs. 3 and 4. × 300.

Figs. 3 and 4. Indicate the rupture of the inner layer of the sheath, so that the cap is held on only by the very thin but very strong outer layer. × 150.

Fig. 5. Shows a larva still in its sheath just after detachment of the cap, which lies beside it. ×150.

Fig. 6. Shows a larva with unusual distension of the cap area and rupture of the inner layer of the sheath, the broken ends of which are seen. ×300.

between their broken ends, together with the swelling of the sheath and the distance between the mouth of the larva and the tip of the sheath, all point to considerable distension of the sheath by increased internal pressure. It is obvious that the cap is here retained in position only by the thin outer layer, which is itself under great tension. It is, however, unusual for the cap to be thus distended. Usually, as is explained below, it is the only part of the sheath that is not distended.

With comparatively low magnifications the break in the inner layer of the sheath is usually visible in most larvae as a refractile ring, indicated in Fig. 2, which runs round the middle of the swollen area. It is along this ring that the cap is eventually detached, by the breaking of the very thin outer layer of the sheath.

It is usually thus detached completely, and is found in the hanging drop beside the empty sheath (Fig. 5); or it may be detached at one side only, in which event the larva withdraws its head from the cap and, pushing this aside,

leaves it still attached to the sheath. Empty sheaths with their caps still attached in this way are often seen and have been figured by others (*e.g.* Veglia, 1915). This method of emergence of the larva through a wide transverse slit at the base of the cap, which gives way, in such instances, along a portion only of its circumference, may perhaps be compared with the longitudinal rent in the sheath in the oesophageal region, through which the fourth larva usually emerges without the detachment of a cap at the third ecdysis.

Ecdyses by the complete detachment of the cap, or by its partial detachment, are both normal. By both these methods the larvae leave their sheaths without difficulty, provided that the greater part of the base of the cap is ruptured and provided that the larvae are active enough. All nematode larvae are more active in acid than in alkaline solutions, and the importance of the activity of the larvae was shown by the fact that, in mixtures containing relatively more NaOH, so that the *p*H was 8 or more, the caps were detached quicker, but the larvae were more sluggish, so that they more often failed to emerge and died partly out of their sheaths. In mixtures at *p*H 6 or 7, containing relatively less NaOH, the changes at the base of the cap took longer, so that the caps took longer to come off, but the larvae were more active, and therefore less often failed to leave their sheaths. It was possible thus to vary to some extent the results by varying the *p*H, due regard being paid to the other factors described below. At *p*H 3 no ecdyses occurred, because, although the larvae were very active, not enough NaOH was present; and the larvae soon died in their sheaths. This applied in fact to all mixtures at a *p*H lower than 6, in which the NaOH had little effect.

Mixtures of stronger solutions of HCl and NaOH (1 and 2 per cent.) caused ecdyses, but they killed the larvae quicker and often shrunk them, being apparently hypertonic to them. In such solutions, at *p*H 9, the ecdyses were effected, not by the detachment of a cap, but by dissolution of the sheaths, as they occur in the stronger dilutions of hypochlorite solutions. This was probably due to the higher concentrations of NaOH in these mixtures. That it was not due to the formation of HOCl in them, is suggested by the fact that when some larvae were put into 0·5 per cent. HCl for 20 hours at 38° C. and then, after being washed in distilled water, were put into 0·4 or 0·8 per cent. NaOH, so that no HOCl could be formed, the sheaths became immediately distended in the NaOH solution, and sometimes were dissolved away. It must be added, however, that none of the larvae treated in this way actually exsheathed, probably because they were not active enough. At room temperature, moreover, 1 per cent. HCl for 18 hours, followed by 8·5 or 10 per cent. NaOH, had no other effect than to cause shrinkage of the larvae. This suggests that the separate action of the HCl and the NaOH, and probably also that of mixtures of them, occurs only at higher temperatures.

That the NaCl which must have been formed in all these mixtures cannot itself produce ecdyses was shown by the absence of ecdyses in solutions of this compound varying from 0·85 to 15 per cent. In mixtures of NaCl and either

$Na_2CO_3$ or NaOH only a few larvae were studied, and all were at room temperature. In mixtures of equal parts of 1 per cent. solutions of NaCl and NaOH the larvae appeared dead but unchanged in 3 hours; and in similar mixtures of 2 per cent. solutions there was considerable shrinkage of the larvae and the sheaths appeared more transparent and the larvae were dead in 6 hours. It was concluded that the sheaths of these larvae had already been altered in the cultures, that in the stronger solutions water was abstracted from the larvae till they were killed and that the salt may also have had some toxic effect, the alteration in the sheaths enabling it to pass through and affect the larvae inside.

Before the larvae emerge they always withdraw their anterior ends from the caps; they never swim away with the detached caps over their oral ends. This is perhaps an adaptation which ensures that they can feed when they are free.

When the cap is detached in these mixtures of HCl and NaOH, the larva is not propelled passively part of the way out of the sheath as it is in Milton, perhaps because the internal pressure does not become so high, or simply because an extensive area of the sheath gives way at the base of the cap, so that the internal pressure is released through a wide opening and has less effect on the larva. It is also to be noted that the detachment of the cap along the ring shown in Fig. 2 releases the widest part of the larva first, namely the posterior part of its oesophageal region. This happens also when ecdyses occur in nature. It is a method more likely to ensure the survival of the greatest number of healthy larvae than the kind of ecdyses that occur in Milton through a relatively small hole at the anterior tip, through which the larvae must be propelled by high internal pressure and their own movements.

All these points are interesting when we remember that the refractile ring shown in Fig. 2 always appears at the same place, that the blistering produced by chlorine water or its vapour is more marked at this point and that distension often first appears here, or is more marked here, as Looss (1911) also noted, when larvae are put into artificial pancreatic juice, which contains at least as much NaOH as the mixtures used for these experiments. It is difficult to resist the conclusion that this part of the sheath, like the cap, may have a slightly different chemical composition and that this difference may have a definite adaptational value, because it ensures, in the manner just indicated, the survival of the greatest number of healthy exsheathed larvae.

## V. THE IMPORTANCE OF THE MOVEMENTS OF THE LARVA

It has already been explained that the bending and swimming movements of the whole larva are essential to its complete emergence from the opened sheath, whether the sheath is opened by the detachment of a cap or by the bursting of its anterior tip. No less important, however, for the successful detachment of the cap are specific movements of the oral end of the larva, which take place *before* the cap is detached.

Many larvae, whose sheaths already showed the rupture of the inner layer

shown in Figs. 3 and 4, had ceased all movement except a slow rotation of their oral ends, which carried the oral ends of the sheaths also with it, around the surface of an imaginary cone the base of which was the refractile ring which indicated the line along which the cap would later be detached. Such rotatory movements, which can be imitated by rotating the finger in a glove so that tension is put on the glove at the base of the finger, would exert tension upon, and therefore would weaken, the sheath at the point where the cap is eventually detached, even if it were not already weakened here by the action of the medium in the manner just described. The tension exerted by these rotatory movements must be increased by the comparative rigidity of the cap itself, which is usually much less distended than the sheath, or not distended at all, so that its diameter may be only a half that of the rest of the distended sheath.

The effect of these rotatory movements on the base of the cap must also be much greater when the oral tip of the cap is fixed by the larva against any solid object, or against such a membrane as the skin or the intestinal mucosa. It is well known that skin-penetrating larvae do thus fix the oral end at right angles to the skin and then lash and bend and rotate their bodies about this fixed point.

Larvae that do not penetrate the skin may, however, execute similar movements, fixing their oral ends against either solid matter in hanging drops or against the surface film, or against sheets of mucosa when they are studied on these. Such movements have been described as boring movements and some writers have supposed that they help ecdyses merely by reason of the friction to which they expose the larva as it passes through the substratum into which it bores. The writer was, however, unable to show that such so-called boring movements had any effect at all on the ecdyses unless the changes described above at the base of the cap had already occurred. Unless these changes have occurred, these so-called boring movements seem, indeed, to be entirely useless to the larva.

Nor is the fixation of the oral ends of the caps, while these rotatory movements occur, necessary for even the detachment of the caps, because many larvae were seen to rupture the sheaths at the base of the caps by these rotatory movements of the oral ends, and also to detach the caps in hanging drops entirely free from any kind of solid or semi-solid matter. Sometimes larvae can be seen executing these rotatory movements with the detached caps still on their oral ends.

These rotatory movements of the oral ends should not be confused with movements of the oral ends of larvae *inside* distended sheaths, which give the impression that the larvae are nosing about in the region of the refractile ring at the base of the caps, as if they were seeking a way out here. Careful observation shows that these movements, unlike the rotatory movements just described, are irregular and have no actual reference to the base of the cap. They can only give this impression when the sheath is so far distended that the oral end of the larva can reach this region of the sheath.

Yet another factor may help to rupture the sheath at the base of the cap. In some larvae, which are to some extent retracted in their sheaths, and whose sheaths behind the cap have laxer folds than usual, it can be seen that these folds travel up over the rigid cap as the larva squirms and coils, much as a piece of india-rubber tubing can be pushed up over itself on to a tap or glass rod. By such movements the base of the cap is buried under the fold of the flexible part pushed over it and later is uncovered again. The frequent repetition of such a movement must also put considerable strain upon the junction of the rigid cap and the flexible folded part of the sheath behind it, and this strain will be applied transversely to the long axis of the larva and practically equally all round the base of the cap. Its action, together with that of the twist applied by the rotation, must powerfully help to determine a break in the sheath at the point where both these forces are applied, namely at the base of the cap, especially if the sheath has already been altered by the reagents here described, or by exposure on the pastures or in the soil, or if it has been often dried or wetted. Larvae can, in fact, be seen in which such a rupture has occurred at one side only, so that when they rotate their heads, the sheaths are seen to gape at one side, and eventually to give way all round. The larvae may, however, emerge by the rupture at one side before this has continued all round the base of the cap, and this no doubt explains why they sometimes appear so to emerge by a lateral slit, as the fourth larvae seem in fact usually to do.

The importance of these larval movements was further emphasised by the fact that the sheaths of some larvae were ruptured by them at the base of the cap, although there was no visible osmotic distension of these sheaths. It may have been present earlier and have collapsed; and there was no means of determining whether any chemical change in the sheath had occurred. Only a few ecdyses were seen to occur like this, namely by the larval movements just described alone; usually they were very slow, requiring 2 or 3 days after the rupture of the sheaths at the base of the caps; and the larvae frequently were not active enough to emerge completely, but died half-way out of their sheaths. Such ecdyses were, moreover, seen chiefly in the mixtures of HCl and NaOH. It seemed clear, therefore, that the osmotic distension of the sheaths is usually necessary for the normal and successful ecdyses.

The length of the cap seems to be always the same in the larvae of each species. It is possible, indeed, that the length of the cap is sufficiently constant to be a specific character. Certainly the cap can usually be distinguished on normal larvae by the fact that it never shows the crinkled appearance characteristic of the rest of the sheath. The transverse folds on the sheath behind the cap may indicate that this part of the sheath is more elastic and perhaps has a slightly different chemical composition, as is suggested by the facts that the cap does not blister and is not distended in the reagents here described. The grooves between the folds perhaps correspond to the transverse striations on the cuticle of most nematode larvae and the folds to the areas of cuticle be-

tween them, some shrinkage of the sheath occurring after it has separated from the larva inside. The writer found that even the Milton solutions which usually cause ecdyses so regularly would not exsheath larvae immediately after they had become mature, but only after a few hours had elapsed. This indicates that some chemical change does occur in the sheath after its separation; some shrinkage of the sheath and the appearance of the transverse folds on it may be the result of this.

Finally a few words may be said about the possible effects on the ecdyses of friction and mechanical damage, because some authors regard these as the most important causes of ecdyses, if not the only ones. The writer's experiments failed to show that these very small larvae could suffer from the effects of friction and mechanical damage, especially in mucoid media similar to those in which they occur in the body. A repetition of many of Looss's (1911) experiments and exposure of larvae to friction and mechanical damage in other ways, indicated that friction and mechanical damage could never be more than a secondary, or even an accidental, cause of ecdyses, and that if these factors did operate, they affected only "older" larvae, or those whose sheaths were already so altered that friction or mechanical damage could affect them. Comparison of the ecdyses of larvae which had been kept in cultures for different periods after they had become mature, that is to say, of what are usually called "older" and "younger" larvae, showed that the "age" of the larvae had no effect upon the ecdyses, but that the sheaths of larvae that had been a longer time in cultures were either more brittle so that they could sometimes be mechanically damaged, though this did not necessarily result in ecdysis, or were chemically changed so that the process described above induced ecdysis.

The conclusion that mechanical damage can rarely alone cause ecdyses is supported by evidence as to the remarkable strength of the very thin outer layer of the sheath by which partially detached caps may often remain attached. Such partially detached caps are often subjected to considerable jolting and displacement as the larvae bend and writhe in media containing solid debris, but they are not detached by this. Attempts to detach them by sucking such larvae up and down pipettes either in water or in mucoid media, or even to remove the partially detached caps with needles, failed. The resistance of the sheaths to the often remarkable distension that occurs in the solutions here described, or in solutions of organic and inorganic sulphides, also demonstrates the strength of this outer layer. It could not possibly be seriously damaged by any friction or other mechanical damage which these very small and light larvae are likely to encounter. The only possible conclusion was that the main factors detaching the caps were the biochemical ones here indicated and also the movements of the larvae themselves. This was confirmed by the fact that so many larvae regularly and completely detached their caps in mixtures of HCl and NaOH free from any solid matter, in which mechanical factors could not possibly operate; and also by the fact that caps were detached completely by larvae which had been inactive for some days and were regarded

as dead. It was clear, therefore, that ecdyses could occur without any mechanical factor of any kind.

## VI. ECDYSES IN THE BODY OF THE HOST

If the explanation of the second ecdysis here suggested is correct, it may be possible to relate ecdyses which occur in the alimentary canal of the host to the acid-base equilibrium in the stomach and duodenum. The writer found that neither saliva, nor the ferments of the gastric and pancreatic juices, could alone induce ecdyses. Ecdyses did not usually occur in artificial gastric and pancreatic juices, in extracts of gastric and duodenal mucosa, in stomach and duodenal contents, in the abomasal contents, or in various other media of this kind. In artificial pancreatic juice, containing NaOH derived from the $Na_2CO_3$ with which it was made up, the sheaths were frequently thinned and swollen as they were in the NaOH and $Na_2CO_3$ solutions described above; and some ecdyses did occur, but could not be regularly obtained. It was concluded that those ecdyses which did occur in artificial pancreatic juice were due to the NaOH it contained, and that they were only effected by larvae whose sheaths had been previously chemically altered by remaining for many weeks in faecal or bacterial cultures. It seemed likely that exposure on the pastures might also effect a similar change, so that larvae so exposed might exsheath when they were put into NaOH alone. This would explain why larvae put by Veglia (1915) into the mouths of experimental sheep, exsheathed there.

A few experiments were done to find out whether the alteration in the permeability of the sheaths described in this paper might be due to hydrolysis of lipoids in the sheaths. Yonge (1932) ascribes the control of the permeability of the lining of the stomach of Crustacea to the lipoids present in it. It seemed at least possible that a mechanism analogous to that described by Yonge might be destroyed by the chlorine and hypochlorite solutions in the experiments described above or by exposure of the larvae on the pastures, which would presumably hydrolyse any lipoids present in the sheaths. We have, however, no evidence that lipoids are present in the sheaths of nematode larvae. Mueller (1928–9) could not demonstrate lipoids in any of the tissues of *Ascaris* which he examined for them. Chlorine would presumably not affect them, if they were present. Chloroform would dissolve them, but the permeability of larvae treated with chloroform appeared not to be affected by it, and Goodey (1922) found that chloroform did not dissolve the sheaths. The writer's treatment of larvae with the lipase obtained from an extract of castor oil bean, did not give any evidence that the permeability of the sheaths had been altered, nor did it cause ecdyses, or render them more frequent. The lipase presumably present in artificial pancreatic juice did not have any effects not attributable to the action of the NaOH also present. It was concluded that not enough had been done to throw any light on any possible part played by lipoids in the sheaths.

The effect of bile, which thins the sheaths and distends them, appears to be

due to the taurocholate in it. In a 1 per cent. solution of sodium taurocholate, and in 1 per cent. solutions of saccharin, or of sodium sulphide, or in $H_2S$ water, or in 0·1 per cent. solutions of cystein hydrochloride in the presence of *B. coli*, which produces some $H_2S$ from this compound, or even in 5 per cent. allyl sulphide (oil of garlic) mixed with water, ecdyses occurred readily, accompanied by changes in the sheaths similar to those seen in mixtures of HCl and NaOH and described above. The sheaths were usually rendered very transparent as they are in NaOH solutions, but they were not dissolved. Distension seemed to be greatest in the $Na_2S$ solutions and the use of these solutions will demonstrate the great tensile strength of the outer layer of the sheath. In watery infusions of chopped garlic bulbs similar ecdyses also occurred, with distension, but the sheaths were rapidly dissolved. The effects of these solutions containing sulphides will be further described in another paper. Their interest here is that they show that sulphides also will alter the permeability of the sheaths with results similar to those produced by weak mixtures of HCl and NaOH. This is interesting in the light of Mueller's (1928–9) conclusion that the cuticle of *Ascaris* is related to the albuminoids, although it does not itself give the reactions of any known albuminoid. If it is related to keratin, and if the larval sheath has a similar composition, this action of sulphides on the larval sheath is not remarkable.

The precise nature of the change in the sheaths produced in the experiments described in this paper requires further investigation by the physical chemist, so that the effect of the various solutions used may be explained.

It would be particularly interesting to inquire whether this were a reaction depending upon dissociation of Cl and OH ions, and, if so, whether the conditions necessary for it occur in the normal stomach, or are more favourable to it in the digestive canal in certain debilitated states, so that these facilitate nematode infections. It is possible that these larvae exhibit a positive chemotaxis to chlorine, and that this may explain why they frequently crawl about actually on the mucosa of the stomach or lie in contact with this. In considering any ionic action that may occur, it should, therefore, be remembered that the environment of these infective larvae may be not so much the stomach contents, as the surface pockets and grooves of the mucosa which is secreting the gastric juice, and, in a ruminant host, is secreting this continuously. Current theory as to the ionic situation during the formation of gastric juice should, therefore, be taken into account.

If it were concluded that not enough alkali were present in the stomach to complete the ecdyses, this would be abundantly present in the duodenum. If the action of the alkali of the duodenal contents were shown to be necessary, following that of the Cl ions in the stomach, we should conclude, perhaps, that parasitic nematodes have been primarily adapted to intestinal life, having to penetrate so far to release themselves from the sheath of the infective stage. Species like *Haemonchus contortus* would then be regarded as secondarily adapted to life in the acid environment of the abomasum, to which their larvae

would have to migrate back after exsheathing in the duodenum. That the primary habitat of nematodes parasitic in the gut was the intestine is also suggested by the fact that the great majority of adult parasitic nematodes seek an alkaline environment, while most of those parasitic in the alimentary canal inhabit the intestine.

Further investigation of the reactions of parasitic nematodes to physical, as well as to chemical, factors in their environment may explain why different intestinal nematode species live in different regions of the intestinal tube. They may also suggest means of rendering the nematode cuticle more permeable to substances poisonous to the nematodes but not toxic to the host, which could be used as anthelmintics.

Whether an explanation similar to that suggested above applies also to the other ecdyses performed by the later parasitic larvae, the writer has not yet determined. The hypochlorite solutions used for inducing ecdyses of the infective larvae had no power to cause the third (first parasitic) larvae to exsheath. The unsuccessful attempts to induce this, the third, ecdysis, together with the behaviour of these first parasitic larvae in various sterile media, will be described in another paper.

## VII. SUMMARY

1. The second ecdyses experimentally produced in 1 in 20 and other watery dilutions of Milton hypochlorite are described. These occur without the detachment of a typical cap. The larva is passively propelled part of the way out through a hole at the anterior tip of the sheath, and completes its emergence by its own movements.

The explanation of this is that the chlorine in the Milton solution alters the chemical composition of the sheath with two results: (*a*) the permeability of the sheath is altered so that increased internal pressure results, by which the larva is propelled out of the hole at the anterior tip, and (*b*) the sheath is rendered soluble in the NaOH in the Milton solution so that it is subsequently dissolved. The larva is expelled through a hole at the anterior tip before the whole sheath is dissolved because the sheath gives way here first to the combined effects of the increased internal pressure and the solvent action of the NaOH, this being its weakest point, where the remains of the mouth of the second larva are.

The larva is only rarely propelled completely out by the raised internal pressure, namely, when the hole at the anterior tip is big enough to allow its widest part, at the base of its oesophageal region, to pass; or, alternatively, when the internal pressure is high enough to force this part of it through the hole. Usually it requires the help of its own movements to force this widest part of it through the hole.

2. Neither chlorine, oxygen, nor caustic soda alone can cause ecdyses, although the latter may dissolve the sheaths of larvae kept long in cultures or exposed long upon pastures, so that a chemical change in their sheaths has

occurred similar to that just described. Caustic soda may then also so weaken and thin the sheaths that larvae, if they are active enough, may break their way out.

Neither carbonic nor hydrochloric acids, nor solutions of sodium chloride, could alone cause ecdyses; nor could solutions of sodium carbonate, although these acted upon "older" larvae in much the same way as solutions of caustic soda, which they must contain.

3. Larvae, however, whether they were recently mature or had been long kept in cultures, showed, when they were treated with chlorine water or with hypochlorite solutions acidified with excess of either HCl or acetic acids, effects which indicated that the conclusion described in (*a*) above was correct. They never exsheathed, but their sheaths were blistered and thinned and frequently distended also, with the exception of the cap which is detached at the ecdysis; this was usually neither blistered, thinned nor distended. This distension was especially marked when these larvae were transferred, after this treatment with chlorine, to solutions hypotonic to them. In hypertonic solutions, on the other hand, the larvae were shrunken. This perhaps explains why larvae are often shrunken away from their sheaths in old cultures from which much evaporation has occurred or when they have been exposed upon pastures.

Their sheaths were, moreover, rendered soluble in 0·4 or 0·8 per cent. NaOH by this treatment with chlorine, so that treatment with chlorine, followed by immersion in weak NaOH, will exsheath the larvae, whether they are active or not, by dissolving off their sheaths. This probably explains why larvae exposed on pastures or kept long in cultures, exsheath sometimes in alkaline media such as artificial pancreatic juice, or in the saliva of sheep.

4. Observation of ecdyses that occurred at 38° C. in about 8 days in mixtures of 0·5 per cent. solutions of HCl and 0·5 per cent. NaOH or in 0·5 per cent. HCl followed by 0·5 or 0·8 per cent. NaOH, showed that these always occurred by the detachment of a typical cap, and that the process was essentially the same as that described above. An alteration of the permeability of the sheath occurred by which the pressure inside it was increased, so that the sheath was often distended. The sheath was at the same time rendered soluble in NaOH.

The cap which was detached at the ecdysis resisted, however, this distension, perhaps because it had a slightly different chemical composition, so that tension existed at the base of this more rigid cap, between it and the more flexible and elastic part of the sheath behind it. The tension at this point was further observed to be increased by specific rotatory movements of the oral end of the larva, which incidentally also determined the length of the cap, and by movements of the more flexible and elastic part of the sheath behind the cap, by which it could be repeatedly rolled up on to the cap and back again, as rubber tubing may be rolled up on to a tap. All these movements, especially when they acted upon a distended sheath, ruptured first the inner layer of the sheath at the base of the cap, and then the much stronger outer layer.

The larva then emerged by its own movements; or sometimes it was propelled out passively part of the way by the release of the high internal pressure.

Both the rotatory movements of the oral end of the larva before the detachment of the cap, and the bending and swimming movements of the whole larva by which it extricates itself from the sheath after the cap has been detached, are essential to successful and normal ecdyses. The rotatory movements of the oral end are probably most effective when the oral tip of the sheath is fixed against some resistant object, as skin-penetrating larvae may, for example, fix it against the skin. This fixation of the oral end is not, however, necessary, because most of the ecdyses here recorded occurred in this way in drops quite free of any solid or semi-solid matter.

5. Some experiments are briefly described which indicate that mechanical damage to the sheaths, or friction applied to them, cannot be more than secondary factors which influence only the ecdyses of so-called "older" larvae, whose sheaths are already chemically altered by climatic factors or long life in cultures, with results similar to those which follow treatment with chlorine.

6. It is suggested that factors similar to those indicated above may be present in the stomach and duodenum of the host and may govern any second ecdyses that occur in these situations; and that changes in the sheaths, similar to those produced by chlorine in the experiments here described, may be produced outside the body of the host, by climatic factors, so that the sheaths become soluble in the saliva or other alkaline digestive juices.

7. All attempts failed to induce the third ecdysis of larvae exsheathed and sterilised by 1 in 20 dilutions of Milton with water, and kept in sterile media until they were ready for this ecdysis. These larvae also, like the infective larvae, were susceptible to changes in the tonicity of the media in which they were kept, so that their sheaths could be alternately distended or collapsed upon them, and the larvae themselves could be shrunken or restored to normal by alterations in the concentration of the media. These third (or first parasitic) larvae showed a remarkable power of resistance to such treatment and seemed to be more resistant to it than infective larvae are.

8. Further investigations of the second ecdyses by biophysical methods may bring all the ecdyses of parasitic nematodes under experimental control and so make possible the artificial cultivation of the adult parasites. They may also suggest methods of altering the permeability of the cuticle of adult nematodes, so that anthelmintics may be found which will penetrate this more readily and at the same time will not be toxic to the host.

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# SOME MORE REMARKABLE CESTODES FROM AMAZON SILUROID FISH

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(With Plates II–IV, containing Figs. 1–34, and Fig. I in the Text)

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## **Endorchis mandubé** sp.n. from **Pseudageniosus brevifilis** Cuv. and Val.

(Figs. 1–3)

I EXAMINED altogether eight specimens of the "Mandubé" (otherwise pronounced "Mandiduber", "Mandober" and "Mandobé" according to locality), a fairly common Siluroid in the Amazon which is easily recognisable on account of the absence of barbels and the short dorsal and elongated anal fins, and which is probably the modern *Pseudageniosus brevifilis* Cuv. and Val. (the *Ageniosus brevifilis* of Goeldi 1898). In the intestine of a specimen, 27 cm. long, of the Mandubé purchased from the market at Manáos I found a single Cestode about 40 mm. long and with a maximum breadth (posteriorly) of 0·79 mm. (in balsam). This Cestode represents a second species of the genus *Endorchis* recently described (Woodland, 1934 *b*). This species is so distinct that it may have to be referred to a new genus, but for the present I include it in *Endorchis*, the definition of which I have modified for the purpose.

The segments of the strobila are numerous and all broader than long (the longest measures 0·36 mm. long and 0·68 mm. broad) and in none of them is the uterus formed. Secondary transverse grooves which are a marked feature in the type species, *E. piraeeba*, are absent. The inconspicuous cirro-vaginal apertures are irregularly alternate and individually situated in the anterior third or quarter of the proglottid length. The scolex (0·7 mm. broad and

about 0·58 mm. long) is simple in form, consisting of a slightly wrinkled continuation of the strobila bearing four large suckers with openings of the form shown in Fig. 1. The scolex is unarmed and bears no apical organ, so differing from the type species. The scolex is only slightly broader than the adjoining strobila which, for the length of more than 3 mm., is unsegmented.

The structure of a mature proglottid (Figs. 2, 3) is as follows. The testes, which lie in two fields in the medulla (often two deep), are about 150 in number and are about $22 \times 11\,\mu$. The cirrus sac is about 0·160 mm. long and 0·044 mm. broad. The cirrus is unarmed and thick-walled. The vagina opens in front of the sac and is only slightly dilated near its opening. The ovary is in two distinct halves and is from one-quarter to one-third of the length of the proglottid. In sections it can be seen that the mass of the ovary lies in the medulla, but that a number of thick dorsal projections pierce the muscle layer and the upper ends come to lie in the dorsal cortex. The vitellaria are very irregular in form and are arranged, in transverse sections, in marginal crescents. Like the ovary, they are partly in and partly outside the medulla, but mainly outside. The longitudinal muscle fibres, apart from the single layer underlying the cuticle, compose a thick band of large bundles. The ventral excretory canals are usually conspicuous, but I have not been able to detect the dorsal pair.

This species is of interest because it is evidently an Endorchidine in process of evolution: the situation of the ovary and the vitellaria, largely and partly medullary respectively, show that it is not as completely evolved as is *E. piraeeba*, the type species. It also retains some traces of Phyllobothriid ancestry, viz. the shape of the sucker openings, the occasional two-deep condition of the layer of muscle bundles and the marginal crescent arrangement of the vitellaria. In all essentials the species belongs to the genus *Endorchis*, if we omit, in the definition of the genus, the references to the size of the scolex and the presence of an apical organ, and I propose to name the species after the local name of the host: *Endorchis mandubé*.

**Anthobothrium mandubé** sp.n. from **Pseudageniosus brevifilis** Cuv. and Val.
(Figs. 4–8)

In another specimen of the Mandubé (38 cm. long), also purchased from the Manáos market, I found, in the anterior intestine, eight medium-sized Cestodes which, on account of the form of scolex, I refer to the genus *Anthobothrium*. This species is of interest because it combines a Phyllobothriid type of scolex with a Proteocephalid type of internal longitudinal musculature.

The largest worm was about 45 mm. long and about 2 mm. broad, midway in strobila length. The segments are numerous and all are broader than long except, in certain cases, the last two or three. A typical gravid proglottid is 1·32 mm. broad and 0·49 mm. long. The marginal cirro-vaginal pores are irregularly alternate, though occasionally unilateral over as many as a dozen segments. The inconspicuous openings are situated about midway in the proglottid length or a little in front, and the vagina opens either behind or in

front of the cirrus sac. Uterine openings, doubtless of the normal slit type, have not developed in my material. The scolex, present in two of my eight examples, is very small, measuring 0·18–0·24 mm. broad and 0·18–0·21 mm. long, and only the projecting phyllidea, bearing trilocular bothridia or suckers, cause the scolex to be slightly broader than the neck. The bothridia (Fig. 4) are typically Phyllobothriid and are rather similar to the trilocular bothridia of species of *Onchobothrium*, but devoid of the hooks. Apical organ, hooks and spines are absent. The neck region is unsegmented, about 0·19 mm. broad behind the scolex, and may be nearly 2 mm. in length.

The structure of a gravid proglottid (Figs. 5–7) is as follows. The testes are 100–150 in number, occupy a single field and measure, in transverse sections, 0·065 × 0·025 mm., being distinctly flattened in form. The cirrus sac, in young gravid proglottids, is about 0·38 mm. long and 0·049 mm. broad, and extends over more than one-fifth of the proglottid breadth in transverse sections. The cirrus is thick-walled and unarmed. The vas deferens forms a relatively small mass of coils outside the sac. The vagina near its opening, direct to the exterior, is only slightly broader than its continuation towards the ovary, and, as already stated, may lie in front of, or behind, the cirrus sac. The ovary is remarkable for its narrowness antero-posteriorly, consisting merely of a thin strip of tissue lying against the posterior border of the proglottid. The uterus bears large lateral diverticula in which eggs are accumulated until the pore is formed. The eggs, as examined in the original formalin-glycerine mixture, are more or less spherical (Fig. 8), are devoid of hooks and measure about 19$\mu$ in external diameter; the embryos measure about 11$\mu$ in diameter. The vitellaria consist of numerous very small follicles situated mostly inside the internal layer of longitudinal muscle bundles where this bends at the proglottid margins; other follicles are situated between the muscle bundles in this region and altogether outside of them. The follicles are very irregular in form and I cannot state their dimensions with any accuracy. The internal longitudinal muscles are very remarkable in their distr butıon when the typical Phyllobothriid form of the scolex is taken into consideration. The fibres form fairly large, rather indefinite, bundles, all arranged in a definite layer which is well separated by a zone of cortical parenchyma from the internal limit of the subcuticula; there are no scattered longitudinal fibres lying between this layer and the outer single layer of fibres underlying the cuticle. In other words, the internal longitudinal muscles are definitely of the Proteocephalid type and, with the exception of a few stray vitellaria and an occasional downward projection of the uterus, they enclose all the genital organs; in short, they have the Proteocephaline arrangement. Dorsal and ventral excretory canals were not seen in my preparations.

The present new species presents one more instance of a form transitional, as regards combination of characters, between the Phyllobothriidae and the Proteocephalidae. Fresh-water Phyllobothriids from Siluroids which, in the few species examined up to the present, have lost the X-shaped or quadripartite ovary, definitely show in this respect one advance towards the Proteocephalid

condition, and I have already described (1934 *a*), in *Myzophorus admonticellia*, a Phyllobothriid which might very well be a precursor of the Endorchidinae in the form of scolex and ovary and in the position of the genitalia in relation to the muscle layer. The present species might likewise be regarded, solely on the position of the vitellaria and the distribution of the longitudinal muscle fibres, as a precursor of the Zygobothriinae, and it is the only instance known to me in which, though the scolex is eminently of the Phyllobothriid type, the internal longitudinal muscle layer is completely Proteocephalid in form. The scolex proves that this worm is a Phyllobothriid (since it is difficult to imagine a Proteocephalid reverting to the Phyllobothriid scolex) and I provisionally refer the species to the only Phyllobothriid genus at present capable of receiving it: *Anthobothrium mandubé*.

**Monticellia piracatinga** sp.n. from **Pimelodus pati** Cuv. and Val.
(Figs. 9–12)

"Piracatinga" is the local name applied on the Amazon to the relatively small Siluroid known to zoologists as *Pimelodus pati* Cuv. and Val. My largest fish measured 50 cm. in length. Out of six specimens examined only one, caught about twenty miles below Manáos, contained the very distinct new species of *Monticellia* now to be described—*M. piracatinga*.

Of this new species I possess two examples, only one of which has fully mature, though not gravid, segments. The mature worm measured in total length about 6 mm., and contained less than fifty segments in the strobila. The last six segments were alone mature and accounted for half of the length of the worm, being, in the case of the last two or three, longer than broad; the terminal segment was 0·84 mm. long and 0·53 mm. broad, and the penultimate segment 0·58 mm. long and 0·53 mm. broad. The genitalia first become completely developed in segments in the anterior half of the worm, and these segments measure about 0·66 mm. broad and 0·19 mm. long. Segmentation of the strobila extends up to the base of the scolex, consequently there is no unsegmented neck. The cirro-vaginal apertures are irregularly alternate and individually open at about a third of the proglottid length from the anterior border. The scolex in both of my specimens has become somewhat distorted in mounting, and I am not able to describe exactly the natural disposition of the surface folds below the suckers, but longitudinal folds are present and are to some extent as portrayed in Fig. 9. The two scoleces measure 0·34–0·36 mm. in breadth and 0·59–0·74 mm. in length, and the long diameter of the suckers varies between 0·16 and 0·22 mm. according to state of contraction. The scolex is unarmed, an apical organ is absent and the central area between the inner edges of the suckers is very small.

The structure of the mature proglottid (Figs. 10–12) is as follows. The testes lie in the dorsal cortical parenchyma, are between 40 and 50 in number, and individually measure, in surface view, about $44 \times 22\,\mu$. The thin-walled cirrus sac extends over about a third of the proglottid breadth, *c.* 0·135 mm. long in

transverse sections, and is narrow (0·033 mm.). The sac contains several coils of the ductus besides the thick-walled unarmed cirrus. The vas deferens forms convoluted coils lying above and for the most part in front of the transverse portion of the vagina. The vagina opens in front of the cirrus sac and both open into a small atrium. The ovary is bulky in the sense that the follicles extend over about a third of the proglottid length. Except for the small median portion, practically the entire ovary lies in the dorsal cortex. The uterus is only present as a thick-walled tube extending up the middle of the proglottid and a pore is undeveloped, though the tube extends down to the ventral subcuticula in the median line. Eggs are not present. The vitellaria are very similar to those of the type species *M. coryphicephala*, consisting of two horizontal rows lying in the lateral cortex, ventral to the ventral excretory canals. The vitellarian follicles are roughly half the diameter of the testes. The ventral excretory canals are large and the dorsal excretory canals very small. The longitudinal muscle system in this species is almost entirely absent and the line of demarcation between medulla and cortex is represented by a thin layer of transverse muscle fibres and partly also by a thin condensation of the parenchyma. Whether this is also the case in the type species *M. coryphicephala* I do not know, because although La Rue (1914) speaks of the "inner longitudinal muscle layer" in that species yet I have not been able to find any description or detailed figures of the layer. In both *M. lenha* (Woodland, 1933 *b*) and *M. megacephala* (Woodland, 1934 *c*), the only other two valid species of *Monticellia*, longitudinal muscle fibres are certainly present, arranged in distinct small bundles.

*M. piracatinga* is the fourth species of *Monticellia* to be described, and it bears a closer resemblance to the type species than either of the other two named above. It is nevertheless a very distinct species, since it differs from *M. coryphicephala* in the following features. The scolex has a broad, long post-sucker region with straight longitudinal folds. A neck is absent, segmentation extending up to the base of the scolex. There are relatively few (under 50?) segments. There are less than 50 testes compared to about 100 in the type species and a much smaller cirrus sac which measures at least 0·286 mm. in the type species.

It appears certain that the type species, *M. coryphicephala*, was discovered in a South American Siluroid.

**Nomimoscolex piracatinga** sp.n. from **Pimelodus pati** Cuv. and Val.
(Figs. 13–16)

Of this species I obtained only three examples and some fragments from the same fish which harboured *Monticellia piracatinga*. Of the three examples one was entire and gravid and the other two immature and devoid of scoleces. The perfect specimen was about 20 mm. in length and 0·7 mm. maximum breadth posteriorly, with a strobila composed of about 50 segments. About three-quarters of the strobila length consists of segments which are longer

than broad, the terminal segment (Fig. 14) measuring 1·77 mm. long and 0·70 mm. in maximum breadth, and the preceding three segments are similar though slightly less in dimension. About a third of the strobila consists of gravid segments. The cirro-vaginal pores are irregularly alternate and individually situated about a third of the proglottid length from the anterior border. The vagina opens in front of the cirrus sac. Uterus pores had not formed in my material. The scolex is small, measures 0·22 mm. broad and 0·19 mm. high (apex to lower edge of suckers), is unarmed, simple in form (Fig. 13) and devoid of an apical organ. The suckers measure 0·106 mm. in external diameter. The unsegmented region of strobila immediately behind the scolex measured 0·26 mm. in breadth and just over 1 mm. in length.

The structure of the gravid proglottid (Figs. 14–16) is as follows. The testes are about 150 in number, situated in one field, and in surface view they individually measure about 40 $\mu$ in diameter. The cirrus sac measures about 0·146 mm. long and 0·109 mm. broad and has very distinct muscles in its wall, especially towards the external aperture. The unarmed cirrus is extruded to some extent in one preparation and is thick-walled and stumpy, measuring 0·109 mm. long and 0·080 mm. wide. The ductus forms a few coils inside the sac. Externally to the sac the vas forms a large mass of the usual convolutions to the cirrus side of the middle of the segment. As already stated, the vagina opens in front of the sac and does not cross the mass of vas deferens coils before it turns back towards the ovary. The ovary is of the form shown in Figs. 14 and 16, and is not more dorsal than ventral in transverse sections of a proglottid. The uterus (Figs. 14, 15) is very narrow, with a pore opening along the entire length of the segment in front of the ovary in completely gravid segments. I did not examine the intra-uterine eggs in the original preserving fluid; in sections they appear to be spherical with an external diameter of 10–11 $\mu$ and an embryo about 8 $\mu$ in diameter. Hooks are absent as usual. In transverse sections of proglottids the vitellaria are arranged as much-curved crescents external to the dorsal and ventral excretory canals. The striking feature about this species is that it is devoid of an internal longitudinal muscle layer, though the usual external subcuticular row of longitudinal muscle fibres is present, and the parenchyma is only demarcated into cortical and medullary regions by a slight condensation of the parenchyma lying on the external surfaces of testes, ovary and uterus. The vitellaria, on the other hand, can be seen to lie in the marginal cortex, *i.e.* external to the lateral continuation of the central parenchymal thickening.

This species must evidently be referred to the subfamily Zygobothriinae (Woodland, 1933 *a*) and the genus *Nomimoscolex* (Woodland, 1934 *b*), the definition of which latter requires to be amended (*vide infra* the note on *N.* (*Proteocephalus*) *lenha*).

**Manaosia braçodemoça** gen. et sp.n. from **Platystoma** sp.
(Figs. 17–20)

In a Siluroid fish, 46 cm. long, called locally "Braço de Moça" ("Maiden's arm"), purchased in the market at Manáos and which, according to Dr J. R. Norman, is probably a species of *Platystoma*, I found portions of a single small Cestode which, though imperfect, is yet sufficiently interesting to warrant a preliminary description. This Cestode consisted of the scolex and 6–7 mm. of attached strobila, the terminal segments not being present. The scolex (Figs. 17, 18) is large, 0·94 mm. in diameter, and globular in form, with a wrinkled surface, which bears the openings of four suckers. Longitudinal sections show that these suckers are large, *c.* 0·33 mm. in diameter, and, in this specimen, entirely hidden inside the spherical scolex. The scolex is unarmed and devoid of an apical organ. An unsegmented neck is present, just over 1 mm. in length and 0·32 mm. wide behind the scolex. The strobila is very opaque with a creased surface, and it is impossible, with the small amount of material at my disposal, to observe the outlines of individual proglottids or other external features. At the end the strobila measured 0·70 mm. in breadth. Transverse sections of two small lengths of the strobila also show clearly only a few portions of the internal anatomy, even such usually conspicuous organs as the cirrus sac and the vagina being very indefinite. Neither a cuticle nor a definite subcuticula could be detected. The only organs I am able to describe briefly are the ovary, the testes, the longitudinal muscle system and the dorsal and ventral excretory canals. The cortical zone of parenchyma has a number of nuclei which appear to belong to a subcuticular layer, and its outer layer has a definite edge but without a cuticle. The longitudinal muscle system consists of a very definite layer of quite large bundles of fibres which is very conspicuous in transverse sections stained with eosin. The medullary area is very small in fully mature segments but larger in relatively immature segments. The ovary (Fig. 20) consists of lobules situated in the medulla, which produce slender processes piercing the dorsal layer of muscle bundles and ending in bulky lobules situated in the dorsal cortex. The organs which I assume to be the testes (Fig. 19) are somewhat similar in shape to the cortical ovary lobules and may be small ovary lobules. My reason for assuming that they are testes is the fact that I cut into transverse sections about 4 mm. of mature strobila: and the ovary cannot extend over the entire proglottid length. I found in some of the sections distinct though minute dorsal and ventral excretory canals. No trace of a uterus was to be seen, and I cannot positively identify vitellaria.

From the foregoing details it seems certain that this Cestode must belong to either the Rudolphiellinae[1] (Amphilaphorchidinae) or the Monticelliinae, and in either case it is necessary to found a new genus since the scolex and possibly the strobilar anatomy are very unlike those of the four genera in

[1] *Vide infra*, p. 223.

these two subfamilies. The provisional definition of this new genus, *Manaosia*, is: Rudolphiellinae[1] or Monticelliinae. Scolex large, containing large suckers embedded in its substance which open on the surface by small apertures. No apical organ. Type species, *Manaosia braçodemoça*.

**Amphoteromorphus parkarmoo** sp.n. from **Pseudopimelodus zungaro** (Humboldt) (?). (Figs. 21–23)

A large Siluroid, measuring 140 cm. in length and 30 cm. broad across the head, caught about twenty miles below Manáos and locally known as the "Pārkarmoo" (phonetic spelling) is the host for the new species of tapeworm now to be described. This fish was greenish yellow on the dorsum and sides and creamy yellow ventrally, and all the barbels were short, the maxillary hardly reaching the gill cleft. Behind the gill cleft was one conspicuous plate. This fish bears some resemblance to the "Pirarará" (*Phractocephalus hemiliopterus*), but it has no obvious plates either on the head or dorsum, such as are present in the latter species. Dr J. R. Norman tentatively identifies this fish as *Pseudopimelodus zungaro* (Humboldt).

In the anterior and middle intestine of this fish I found eight more or less entire tapeworms and about half a dozen small immature specimens. The longest measured about 33 mm. in length without terminal segment, and about 1 mm. in maximum breadth. These tapeworms represent a new and third species of *Amphoteromorphus*, the two other species being the type, *A. peniculus* Diesing 1850, and a very distinct species, *A. piraeeba*, recently described (Woodland, 1934 *b*). The present species of *Amphoteromorphus*—*A. parkarmoo*—in most respects resembles *A. peniculus*.

The scolex varies in shape according to whether it is attached to the mucosa or free (Figs. 21, 22), and in size according to the age of the worm. In the unattached condition the four bilocular suckers are well exposed on the sides of an anterior terminal pyramidal projection, which is continuous with a basal portion with deeply creased walls; in the attached condition the base is contracted into a kind of flat cushion, on the upper surface of which the four bilocular suckers lie and the pyramidal projection becomes more or less flattened out. I have described these forms of the scolex because they differ from those of the scolex of the type species *A. peniculus* (Woodland, 1933 *a*), though the scoleces of the two species are in general very similar. Another structural difference from the scolex of the latter species is the entire absence of long or short processes covering the terminal area. As in *A. peniculus*, the scolex is unarmed and there is no apical organ. In size the scolex varies, according to size and age, in breadth from 0·53 to 1·12 mm. and in length from 0·47 to 0·88 mm. In the strobila all the segments are broader than long, the longest, in proportion to breadth, measuring 0·59 mm. long and 0·94 mm. broad. There is, in extended specimens, a short unsegmented neck region measuring 1·3 mm. in two mounted specimens, but in contracted specimens

[1] *Vide infra*, p. 223.

the segmentation extends up to the scolex. The cirro-vaginal apertures are unilateral, on the right side of the strobila and individually situated very near the anterior border of the proglottid.

Internally the proglottid structure is typical of the genus. The testes are, as in the type species, less than 40 in number, occupy a single field, and measure individually about $40 \times 30 \mu$. In transverse sections of young gravid proglottids the cirrus sac measures $0{\cdot}256 \times 0{\cdot}036$ mm., extending across about one-third of the proglottid breadth; in older gravid proglottids it extends nearly half-way across, is thin-walled and contains ductus coils as well as the unarmed cirrus. The vas forms the usual convoluted mass outside the sac. The vagina opens in front of the cirrus sac. The ovary and uterus are similar to those of the type species. The vitellaria only differ from those of the type species in that the ventral rows do not extend so far inwards towards the mid-ventral line. Intra-uterine eggs (Fig. 23), when examined in formalin, are seen to be similar in form to those of the type species but are somewhat smaller, the hookless embryos measuring only about $12 \mu$ in diameter, and the outer shell about $22 \times 16 \mu$. The medullary parenchyma, as in the type species, is much more dense in texture than the cortical, and the longitudinal muscle layer is similar. Dorsal and ventral excretory canals are present, though inconspicuous, but I have not been able to detect the transverse connecting vessels or the foramina secundaria found in the type species.

Despite the resemblances between this species and the type species, I believe that the two are distinct, though many of the differences are indefinable. One of the chief distinctions lies in the difference in form of the scolex. In *A. peniculus* the scolex is either so contracted at the apex as to hide the bilocular suckers or expanded so as to expose them freely and the hair-like processes borne by the cuticle. In the present species the suckers are always freely exposed both in the unattached and attached forms and hair-like processes are absent. Other minor differences are the shorter ventral rows of vitellaria, the absence of transverse excretory canals and foramina secundaria and the smaller eggs.

**Nomimoscolex (Proteocephalus) lenha**: A CORRECTION AND JUSTIFICATION (Fig. 24)

In a previous paper (Woodland, 1933 *b*) I described, under the name *Proteocephalus lenha*, a new species of Cestode from the Amazon Siluroid *Platystomatichthys sturio* (the "Lenha"). I failed to notice at the time that in this species the vitellaria are external to the longitudinal muscle layer, *i.e.* in the lateral cortex (see my Fig. 19, Pl. XXXI in the paper referred to, and Fig. 24 of the present paper), are arranged in the form of small very curved crescents, *i.e.* not in the usual Proteocephaline manner as previously stated, and in consequence the species cannot be referred to *Proteocephalus* but should be placed in the Zygobothriinae (Woodland, 1933 *a*) and the genus *Nomimoscolex* (Woodland, 1934 *b*): *N. lenha*. The type species of the genus *Nomimoscolex* is

*N. piraeeba*, and the present species chiefly differs from it in the greater length of the strobila, in the scolex being devoid of an apical organ, in the marginal crescents of the vitellaria assuming the form of small horseshoes, and in the larger number of testes (more than 200 as compared with 100–150). The definition of the genus *Nomimoscolex* must be amended as follows to include the present species and *N. piracatinga* described above (p. 211): Zygobothriinae. Scolex of small size, with normal suckers, apical organ present or absent; irregularly alternating cirro-vaginal pores; vitellaria marginal, as slightly curved crescents or in horseshoe form.

In view of the fact that I at first referred *N. lenha* to the Proteocephalinae, it may be objected that the mere external position of the marginal vitellaria to the lateral longitudinal muscles in *Nomimoscolex* and their internal situation in *Proteocephalus* do not constitute a difference sufficiently marked to justify the placing of these two genera in separate subfamilies, and Harwood (1933) points out that in four species of typical Proteocephalines from snakes the vitellaria are described, by three authors, as lying external to the longitudinal muscle layer. But in these latter cases the external position merely consists of the very narrow strand of vitellaria having become slightly shifted outwards from among the few scattered longitudinal muscle fibres found towards the sides of the segment: the vitellarian strand is not conspicuously external to a well-defined medullary area, as it is even in *Nomimoscolex lenha*. Moreover, even if transitional conditions do occur, these afford no argument against the Zygobothriinae being regarded as a subfamily as well founded as the other seven subfamilies of the Proteocephalidae, all of which exhibit easily distinguishable arrangements of the principal genital organs relative to the longitudinal muscle band. Transitional arrangements must have occurred in the evolution of these forms and indeed still occur. Harwood (1933), however, questions altogether the "fundamental" nature of the "monticelliid character", *i.e.* the situation of some of the genitalia external to the longitudinal muscle layer, and concludes that "the relative position of the internal organs and the inner longitudinal muscle sheath is a character of no more than specific importance". He considers, with Southwell, that the form of the scolex affords a safer guide to true taxonomic affinity, as in the case of the "Corallobothrium" form of scolex found in species of *Corallobothrium*, *Ephedrocephalus* and *Goezeella*. Harwood's arguments are that the longitudinal muscle system is very variable in development, even in the same species, and that "it is as easy to regard the internal organs as stationary and the muscle layer as the mobile organ". However, it is not a case of mere degree of development of the muscles but of position relative to the other organs, especially massive organs like the ovary and testes, and this is a character as constant as any other character. Furthermore, the internal organs are not stationary; for example, the ovary and testes in Monticelliinae are certainly more dorsal than the same organs in most Proteocephalinae. Harwood's arguments therefore do not appear to me to carry much weight, and his case would have been more

convincing had he quoted such facts as those recorded by Wardle (1932) who describes three species (said to be closely related) of *Eubothrium* (Amphicotylid Dibothriocephalids) in which the vitellaria are in a different position in each species. In *E. crassum* the circle of vitellaria is clearly external to the muscle layer; in *E. salvelini* the vitellaria are mingled with the muscle bundles, and in *E. rugosum* the circle of vitellaria is definitely inside the muscle layer. In this instance, either the migration of vitellaria is clearly of very little morphological importance or these three species should not be grouped in one genus. For the present I maintain that even if, as Harwood says, the "monticelliid character" is unassociated with any conceivable function, it is more likely to prove a valid guide to affinity than the particular form of an external organ of attachment, because similar conditions of attachment might produce such an organ independently in distantly related species.

### **Nomimoscolex kaparari** sp.n. from **Pseudoplatystoma tigrinum**
(Figs. 25–28)

*Pseudoplatystoma tigrinum*, the "Kaparari" of native fishermen on the Amazon, has already been given by Diesing (1850, 1855) as the host for the curious tapeworm *Peltidocotyle rugosa*, and I have recently described (Woodland, 1933 *a*, 1934 *b*) another new Cestode from this fish, viz. *Spatulifer surubim* (now regarded as *Monticellia surubim*; *vide infra*, p. 222), which I at first mistook for Diesing's species just named. In three other examples of this fish, captured between Manáos and Santarem, I found a third species of Cestode which I will now describe.

Altogether I possess six examples of this new worm, only one of which, the largest, is devoid of a scolex. The largest worm, less the scolex but including the neck, measured about 30 mm. in length, with a maximum breadth of 0·996 mm. (in balsam), and was the only fully mature worm. The strobila is fairly uniform in breadth, the last third or quarter consisting of gravid segments; it only tapers anteriorly and consists of less than 100 proglottids. All the proglottids, save the terminal, are broader than long, the longest measuring 0·78 mm. long and 0·94 mm. broad. The terminal proglottid only differs from the preceding by being slightly longer than broad and possessing a rounded posterior contour and somewhat elongated ovaries. The cirro-vaginal openings are irregularly alternate and situated individually in the anterior third of the proglottid length. The uterine openings are elongate slits extending over the whole of the proglottid in front of the ovaries.

The scolex (Fig. 25) is of peculiar form, the anterior surface constituting a flattened dome which is very constant in shape and apparently consists largely of some kind of cornified tissue. The margins of the suckers also appear to be somewhat rigid and they carry very distinct triangular projections, one at each of the two upper corners of each sucker, eight in all. These projections must be composed of thickened cuticle and are stratified, but, though claw-like, cannot

be described as hooks. The suckers are thus of peculiar form and remind us of some Phyllobothriid types. I cannot detect an apical organ in my whole-mount preparations. The scolex (five specimens) is 0·28–0·33 mm. broad and 0·149–0·26 mm. long. An unsegmented neck is present 0·83–1·16 mm. long and, immediately behind the scolex, is of the same breadth as the scolex base.

The structure of a gravid proglottid (Figs. 26–28) is as follows. The testes are under 100 in number, lie in a single field above the uterus and internal to the layer of internal longitudinal muscles, and measure about $0{\cdot}055 \times 0{\cdot}036$ mm. The cirrus sac has a muscular wall and is large, measuring in transverse sections 0·237 mm. long and about half this breadth. The unarmed cirrus is long and thick-walled, and the ductus forms several coils inside the sac; outside the sac the vas forms a mass of convolutions lying towards the middle of the proglottid. The vagina opens behind the sac, both opening into an atrium, and the vagina appears to be convoluted between its opening and the middle of the segment. The ovary is massive and is wholly internal to the inner longitudinal muscle layer. The uterus diverticula are rather small, usually only extending laterally about half-way to the proglottid margin. The uterus pore is wide. The vitellaria, unlike the testes and ovary, lie external to the inner longitudinal muscle layer, and form flattened, rather elongated, crescents in transverse sections. The vitellaria measure about $0{\cdot}040 \times 0{\cdot}018$ mm. I omitted to examine the eggs in the original formalin-glycerine mixture, but in sections they are spherical with an external diameter of about $14{\cdot}6\mu$, the diameter of the hookless embryo in balsam being about $9\mu$. The usual dorsal and ventral excretory canals are present, though the latter are not always distinguishable. The longitudinal muscle system consists of a very pronounced subcuticular layer of stout fibres, often two or three deep, and an internal layer of small bundles of 7–8 larger fibres situated immediately internal to the inner edge of the subcuticula, *i.e.* with little or no parenchyma separating the two.

The longitudinal muscle system, though, like the scolex, retaining Phyllobothriid features, may yet be regarded as of the Proteocephalid type, and the same may be said of the scolex and the vitellaria. Assuming that the worm is a Proteocephalid, it evidently belongs to the Zygobothriinae, the only subfamily in which the vitellaria alone are situated external to the inner layer of longitudinal muscle bundles. Of the three genera contained in the Zygobothriinae, viz. *Zygobothrium*, *Amphoteromorphus* and *Nomimoscolex*, the last genus is the only one capable, with but slight further emendation, of receiving the present species—*N. kaparari*. I am aware that the scolex of the present species differs widely from the scoleces of other species of this genus in possessing the shield-like end-piece and stiff lappets on the upper margins of the suckers, but I am averse to founding new genera solely on scolex characters unless these are very fundamental in nature.

**Monticellia rugosa** sp.n. from **Pseudoplatystoma fasciatum** (Linn.)
(Figs. 29–33)

The "Sudobīm" (or "Zurubīm", the "Sorubīm" of Goeldi, 1898) is a very common Siluroid in the Amazon (I examined altogether twenty specimens, eleven of which were infested with Cestodes), the zoological name of which is *Pseudoplatystoma fasciatum* (Linn.). This fish must be distinguished from the "Surubīm" of Goeldi, otherwise called "Kaparari" by the native fishermen, a rarer fish, which is the modern *P. tigrinum* Cuv. and Val.

In two examples of the Sudobim I found the very distinct new species—*Monticellia rugosa*—now to be described. *M. rugosa* is the fifth known species of the genus, the others being the type species, *M. coryphicephala*, and the three species previously described (Woodland, 1933 *b*, 1934 *c*, and in the present paper), viz. *M. lenha*, *M. megacephala* and *M. piracatinga*. *M. rugosa* is easily distinguished from the other three species by the large peculiar scolex which very closely resembles those of species of *Peltidocotyle*.

My largest examples of *Monticellia rugosa* out of about two dozen specimens measured about 40 mm. in length, with a maximum breadth of about 1 mm. posteriorly. Only about six specimens were gravid, the remainder being mature only or immature. Gravid segments may, when extended, be twice as long as broad, but the majority are either very little longer than broad or square or broader than long. The terminal proglottid only differs from those preceding in being proportionately longer and in possessing a rounded posterior border and elongated ovaries. The cirro-vaginal pores are irregularly alternate and individually are situated in the anterior fifth or quarter of the proglottid length. The uterine pore is the usual median longitudinal cleft. The scolex (Fig. 29) is relatively very large (0·6–1·0 mm. broad and 0·88–1·3 mm. long) and consists of a conical crown, slightly wrinkled longitudinally, bearing four conspicuous suckers with a maximum diameter about $0{\cdot}29 \times 0{\cdot}19$ mm., and a broader thick-cuticled elongated base, deeply marked with longitudinal furrows. This base, which is usually very long but occasionally contracts to quite small dimensions, merges into the segmented strobila, an unsegmented neck being absent. An apical organ is absent and likewise hooks and spines.

The structure of a gravid proglottid (Figs. 30–33) is as follows. The testes are numerous, between 150 and 200 in number, occupy a single field in the dorsal parenchyma and measure, in transverse sections, about $0{\cdot}065 \times 0{\cdot}55$ mm. The cirrus sac is large and very broad for its length ($0{\cdot}28 \times 0{\cdot}18$ mm. in surface view) and extends over about one-third of the proglottid breadth. The wall of the sac is rather thick and contains muscle fibres. The sac contains a thick-walled cirrus and several coils of the ductus. The vas forms as usual a mass of coils lying near the middle line of the segment. The vagina lies, and opens, behind the cirrus sac. The ovary is very massive with individual follicles very indistinct, and lies in the dorsal cortical parenchyma. The uterus diverticula are numerous and not very elongated, the breadth of the entire uterus being

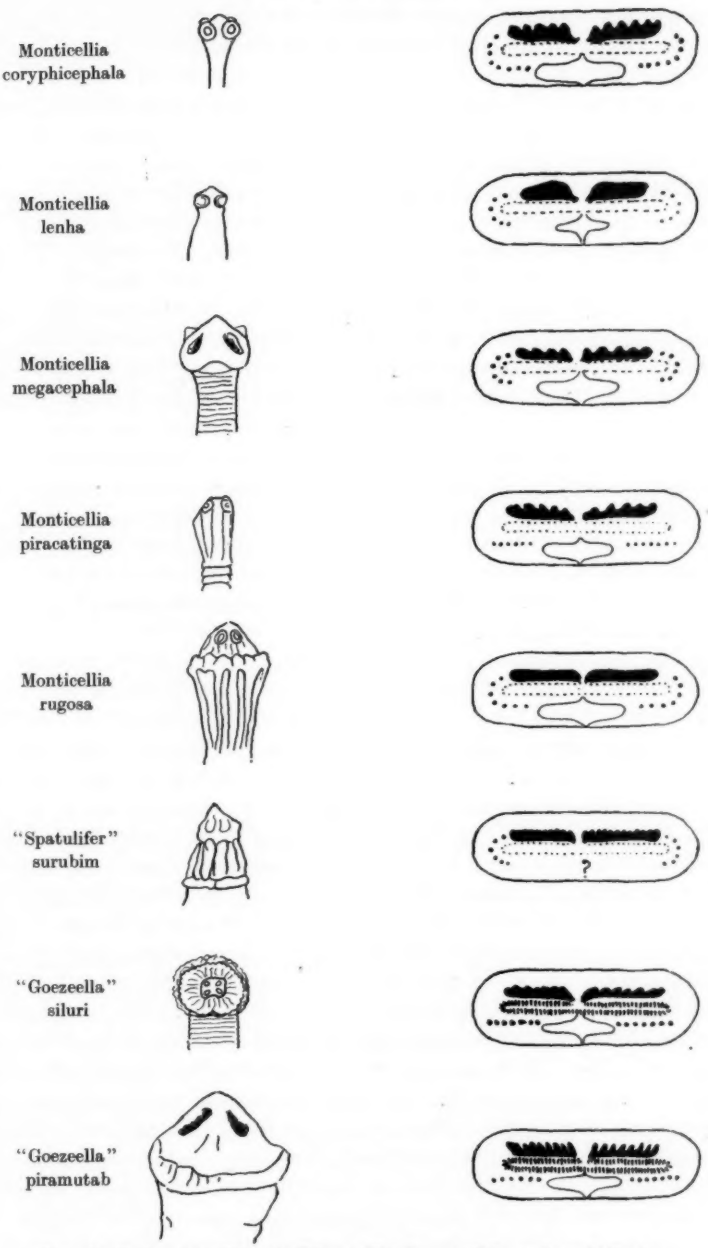
about half that of the entire unextended segment, and they extend over nearly the whole length of the segment, lying also ventral to the ovary. I omitted to examine the eggs in the original preserving fluid, but in sections they appear to be approximately spherical, the external shell measuring, in balsam, about 14·6 $\mu$ in diameter and the hookless embryo about 11 $\mu$. The vitellaria are globular bodies measuring 0·025–0·05 mm. in diameter, and consisting of small follicles measuring about 0·007 mm. They are situated in the marginal cortex and form crescents in transverse sections, the upper arm of each being much shorter than the ventral: in other words, they are mainly ventral, only extending a little distance on to the dorsal surface. Dorsal and ventral excretory canals are present, both being very minute, and the small ventral canals communicate with the exterior on the ventral surface by means of a pair of foramina secundaria in each segment in the region of the ovary. The longitudinal muscle system, as is usual in species of *Monticellia*, is not strongly developed. The small fibres are fairly numerous, especially at the margins, but though thickly clustered in places, distinct bundles can hardly be said to exist: the fibres are merely massed together at the margins, and elsewhere they form layers one or two or more fibres deep.

### The species of the Monticelliinae and *Spatulifer surubim*

I have named the species just described *Monticellia rugosa* despite the fact that its scolex is of a very different type from those possessed by the four other known species of *Monticellia*, and I propose to justify this nomenclature. In addition to the five known species of *Monticellia*, the subfamily of the Monticelliinae includes the two known species of Fuhrmann's genus *Goezeella*, and I am strongly inclined to add the species which I first described as *Peltidocotyle rugosa* (Woodland, 1933 *a*) and later (Woodland, 1934 *a*) named *Spatulifer surubim* and then referred provisionally to the "Amphilaphorchidinae". I now consider it highly probable that the uterus of this last species will be found to lie outside the longitudinal muscle layer in the ventral cortex and, if this proves to be the case, the species is of the *Monticellia* type, all other features of its anatomy agreeing with this view. If we include *Spatulifer surubim*, the subfamily Monticelliinae will contain in all eight[1] species up to date.

Among these eight species the only features known to me which are at all useful for generic distinction are the form of the scolex, the distribution of the vitellaria, and possibly the degree of development of the internal longitudinal muscle layer (see Text-fig. I). As regards the form of the scolex, in *Monticellia coryphicephala* and *M. lenha* this is as simple as possible; in *M. megacephala*, each of the four suckers is situated on a lobe of the scolex, the edge of which is capable of being turned forwards, and in *M. piracatinga* the region below the scolex is elongated and has simple longitudinal folds; in *M. rugosa* and *Spatulifer surubim*, the elongation of the scolex and folding of the wall

[1] I have since discovered a ninth species—*Monticellia spinulifera*—in *Pseudoplatystoma fasciatum* (Linn.), an account of which I have recently published (Woodland, 1935).




Text-fig. I. Species of Monticelliinae. The figures of the scoleces indicate roughly the relative sizes of the actual scoleces.

below the suckers have greatly increased; in the two species of *Goezeella* the edge of the area bearing the suckers has expanded and forms a conspicuous wrinkled collar. We obviously cannot create a genus for each of these three or four types of scolex, though we might if each scolex type were associated with a distinct type of vitellaria distribution, but this is not the case. *Monticellia lenha*, *M. megacephala* and *Spatulifer surubim* have typical vitellaria crescents (in transverse sections), while in *Monticellia coryphicephala* and *M. rugosa* the "crescents" have spread ventrally and diminished dorsally, and in *M. piracatinga* and the two species of *Goezeella* the vitellaria are solely ventral. It is true that the two species of *Goezeella* have longitudinal muscle layers very much thicker than those of other species, but we can hardly employ this as a generic character when we realize that a true *Monticellia* with a thick layer may be discovered at any time and that *M. megacephala* and *M. rugosa* are more or less intermediate between *Goezeella* species and the other species of *Monticellia* which possess either very thin layers or hardly any muscles at all.

Groupings of the two principal characters thus being impossible, the only alternative is to label all the eight species *Monticellia* and so to regard their differences as only of specific value. There is the less objection to this course since the number of species is but small; only in such huge genera as *Proteocephalus* is it admissible to employ small differences of scolex form for founding subgenera. If my proposal be accepted, it follows that the genera *Goezeella* and *Spatulifer* must disappear.

### Note on "*Ephedrocephalus*" *lobosus* (Riggenbach 1896) and the Ephedrocephalinae, Rudolphiellinae and Amphilaphorchidinae

"*Ephedrocephalus*" *lobosus*, first described by Riggenbach in 1896 from *Pimelodus pati* Valenc. (from the Rio Paraguay, South America) under the name of *Corallobothrium lobosum*, was referred by Mola in 1906 to Diesing's genus *Ephedrocephalus*, but later Fuhrmann (1916) created a new genus *Rudolphiella* to receive it, on account of the considerable anatomical differences from *Corallobothrium solidum* Fritsch. Later still, however, Fuhrmann and Baer (1925) followed Mola (1906) in regarding this worm as a second species of *Ephedrocephalus*, the type species being *E. microcephalus*, and this is the current view.

Recently, on reading Fuhrmann's account of the anatomy of this species, it has become evident that it is almost identical with that of the two species recently described, viz. *Amphilaphorchis piranabu* and *A. myoides* (Woodland, 1934 *a*). In all three species the scoleces are similar, the testes are cortical and, in transverse sections, have the peculiar feature of turning round ventrally, *i.e.* overlapping at the margins; the vitellaria are cortical and ventral, the uterus is medullary, the dorsal ovary is partly cortical and partly medullary, and the hosts are from the same zoogeographical region. It is therefore clear that *Amphilaphorchis* becomes a *nomen nudum*. On the other hand, Mola and Fuhrmann are mistaken in regarding the species *lobosus* as a second species of

*Ephedrocephalus*, since *E. microcephalus*, the type species, differs in several important particulars, viz. the ovary in this species (*E. microcephalus*) is purely medullary, the thick layer of longitudinal muscle bundles being apparently impenetrable (Fig. 34), the vitellaria are, except in the middle line, continuous across the ventral surface of the longitudinal muscle band, and the testes do not overlap ventrally at the proglottid margins. Since, according to my scheme of classification of the Proteocephalidae, the purely medullary situation of the ovary in *E. microcephalus* and the partly cortical situation of the ovary in *lobosus* and "*Amphilaphorchis*" *piranabu* and "*Amphilaphorchis*" *myoides* constitute a fundamental difference, it is necessary to remove *lobosus* from the Ephedrocephalinae and to include this species, with the two "*Amphilaphorchis*" species, in Fuhrmann's genus *Rudolphiella*, which must therefore be re-established, the subfamily Amphilaphorchidinae becoming the subfamily Rudolphiellinae (Art. 5 in *Rules of Nomenclature*).

## SUMMARY

A second species of the genus *Endorchis* is described—*E. mandubé*; also new species of the rare genera *Monticellia*, *Amphoteromorphus* and *Nomimoscolex*, viz. *Monticellia piracatinga*, *M. rugosa*, *Amphoteromorphus parkarmoo*, *Nomimoscolex piracatinga* and *N. kaparari*.

A new genus *Manaosia* is created to contain the anomalous species *M. braço de moça*. *Proteocephalus lenha* becomes *Nomimoscolex lenha*.

The species *Spatulifer surubim*, originally described by me as *Peltidocotyle rugosa* and later referred to the Amphilaphorchidinae, is now regarded as a species of the Monticelliinae.

The eight known species of the Monticelliinae are all referred to the single genus *Monticellia*, since, differences in vitellaria distribution not corresponding to differences of scolex form, the only alternative is to create an unnecessary number of genera. The genera *Goezeella* Fuhrmann and *Spatulifer* Woodland are thus regarded as superfluous.

"*Ephedrocephalus*" *lobosus* is removed from the Ephedrocephalinae (which retains its single species *E. microcephalus*) and, with the two species of "*Amphilaphorchis*" recently described by me, is placed in Fuhrmann's genus *Rudolphiella*, now re-established, in the subfamily Rudolphiellinae, thus replacing the Amphilaphorchidinae.

The complete list of known subfamilies, genera and species of the Proteocephalidae described from the Amazon, amended in accordance with the above conclusions, has been published in my last paper on the subject (Woodland, 1935).

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## EXPLANATION OF PLATES II–IV

### PLATE II

*Endorchis mandubé* sp.n.

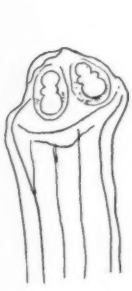
Fig. 1 (×27): the scolex.
Fig. 2 (×87): transverse section of proglottid anterior to ovary.
Fig. 3 (×87): transverse section of proglottid in region of ovary.

*Anthobothrium mandubé* sp.n.

Fig. 4 (×87): the scolex.
Fig. 5 (×39): ventral aspect of gravid proglottid (the intra-uterine eggs are not shown).
Fig. 6 (×56): transverse section across one-half of a gravid proglottid in region of ovary.
Fig. 7 (×39): transverse section of gravid proglottid anterior to ovary.
Fig. 8 (×395): intra-uterine egg (sketched in formalin).

*Monticellia piracatinga* sp.n.

Fig. 9 (×56): the scolex.
Fig. 10 (×56): dorsal aspect of mature proglottid.
Fig. 11 (×87): transverse section of proglottid anterior to ovary.
Fig. 12 (×87): transverse section of proglottid in region of ovary.



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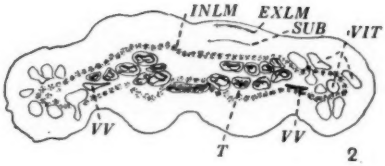


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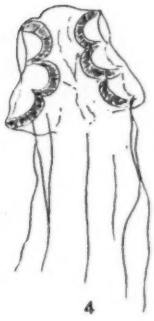

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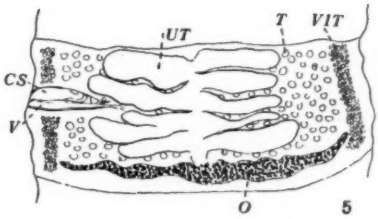


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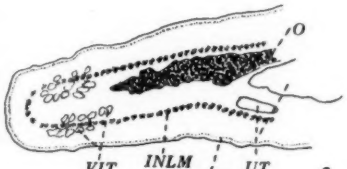


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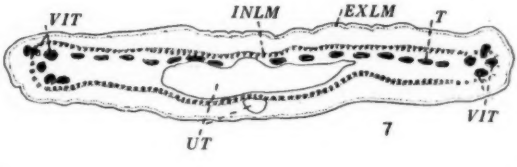


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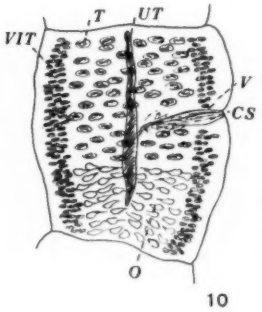


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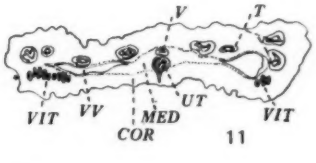


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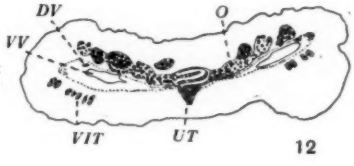


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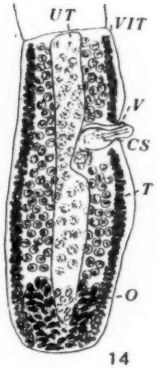

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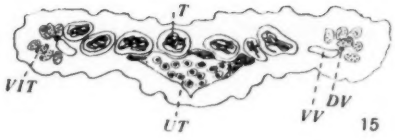

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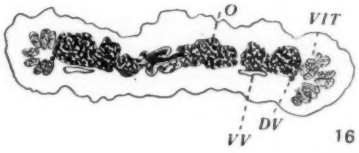

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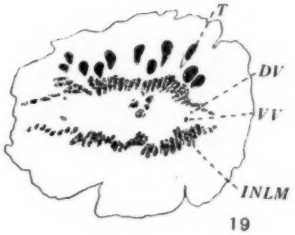

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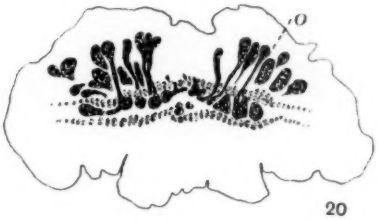

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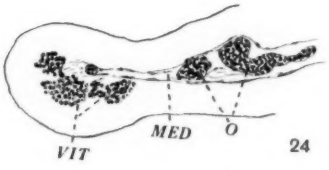

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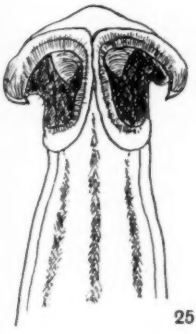
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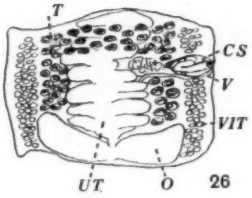

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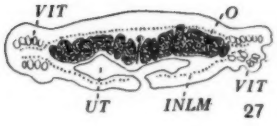

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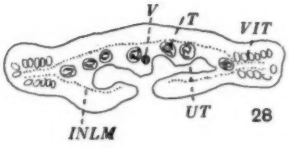

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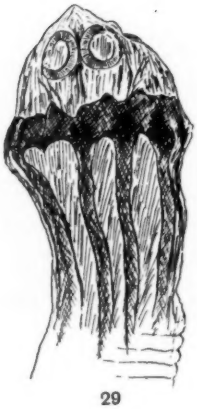
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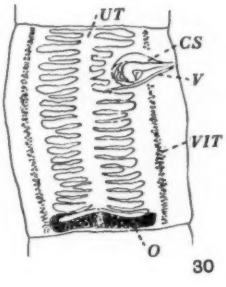

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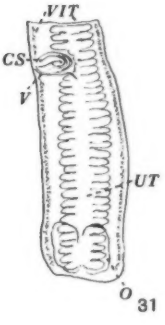

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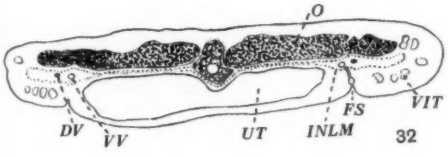

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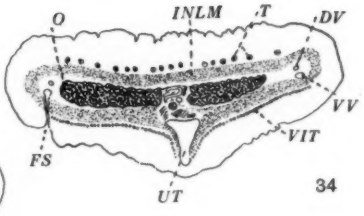

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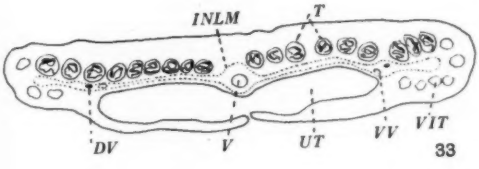

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## PLATE III

*Nomimoscolex piracatinga* sp.n.

Fig. 13 ( × 56): the scolex.
Fig. 14 ( × 27): ventral aspect of terminal proglottid.
Fig. 15 ( × 87): transverse section of proglottid anterior to the cirrus sac (parenchyma not shown).
Fig. 16 ( × 87): transverse section of proglottid in region of ovary.

*Manaosia braçodemoça* gen. et sp.n.

Fig. 17 ( × 17): the scolex seen as an opaque object.
Fig. 18 ( × 27): longitudinal section through the scolex to show the large internally placed suckers.
Fig. 19 ( × 87): transverse section of proglottid anterior to ovary.
Fig. 20 ( × 87): transverse section of proglottid in region of ovary.

*Amphoteromorphus parkarmoo* sp.n.

Fig. 21 ( × 17): scolex in unattached condition.
Fig. 22 ( × 17): scolex in attached condition.
Fig. 23 ( × 395): egg (sketched in formalin).

*Nomimoscolex* (*Proteocephalus*) *lenha*

Fig. 24 ( × 87): transverse section of half a proglottid in region of ovary to show the cortical situation of the vitellaria.

## PLATE IV

*Nomimoscolex kaparari* sp.n.

Fig. 25 ( × 87): the scolex (in outline).
Fig. 26 ( × 39): dorsal aspect of a gravid proglottid (the testes are not shown in the middle area save anteriorly).
Fig. 27 ( × 39): transverse section of gravid proglottid in region of ovary.
Fig. 28 ( × 39): transverse section of gravid proglottid anterior to ovary.

*Monticellia rugosa* sp.n.

Fig. 29 ( × 27): the scolex in side aspect.
Fig. 30 ( × 27): a gravid proglottid of normal shape (testes not shown).
Fig. 31 ( × 17): elongated gravid proglottid in outline (testes not shown).
Fig. 32 ( × 56): transverse section of gravid proglottid in region of ovary (intra-uterine eggs not shown).
Fig. 33 ( × 56): transverse section of gravid proglottid anterior to ovary (eggs not shown).

*Ephedrocephalus microcephalus* Diesing 1850

Fig. 34 ( × 17): transverse section of gravid proglottid in region of ovary to show the entirely medullary situation of the latter, the thick longitudinal muscle layer and the disposition of the vitellaria along the entire ventral surface, except in the middle line.

Lettering to figures: *COR*, cortex; *CS*, cirrus sac; *DV*, dorsal excretory canal; *EXLM*, external longitudinal muscle layer; *FS*, foramen secundarium; *INLM*, internal longitudinal muscle layer; *MED*, medullary area; *O*, ovary; *SUB*, internal limit of subcuticula; *T*, testes; *UT*, uterus; *V*, vagina; *VIT*, vitellaria; *VV*, ventral excretory canal.

(*MS. received for publication* 12. IX. 1934.—Ed.)

# A NEW CILIATE, *ENTODINIUM BOVIS* N.SP., FROM THE STOMACH OF *BOS TAURUS* L., WITH THE REVISION OF *ENTODINIUM EXIGUUM*, *E. NANELLUM*, *E. SIMPLEX*, *E. DUBARDI DUBARDI* AND *E. PARVUM*

By P. WERTHEIM, M.Sc., Ph.D.
*Zoological Institute, University of Yugoslavia, Zagreb*

(With 6 Figures in the Text)

## Description of *Entodinium bovis* n.sp.

Large populations of the new species of *Entodinium* were found in 27 out of 120 stomachs (from the paunch to reticulum) of *Bos taurus* from various regions of Yugoslavia. The stomach content was fixed in 4 per cent. formaldehyde. Unstained glycerine mounts and material stained in iron haematoxylin and Mallory's phosphor-molybden haematoxylin were used. Twenty individuals from each population, *i.e.* 540 individuals in all, were measured. The length was found to vary from 26·25 to 38·5$\mu$, and the width from 24·5 to 31·5$\mu$. The relation between the length and the width was 1·16, which indicates the average degree of rotundity. This rotundity, especially noticeable in individuals in which the adoral membranelle zone is retracted, may sometimes be less pronounced at the anterior end. *Entodinium bovis* is considerably flattened, so that its thickness does not exceed 6–10$\mu$. The pharynx is curved dorsally. The cuticle is longitudinally striated. The ectoplasm is more developed in the anal region than laterally. The macronucleus is elongated, 10·5–21 × 3·5–5·25$\mu$, and is thicker at its anterior end. The micronucleus is round and lies ventral to the macronucleus at a point about the middle of its length. The contractile vacuole lies ventral to the macronucleus, near to its anterior end. The anus is usually situated in the middle of the posterior end of the ciliate, though sometimes it lies slightly ventral.

No other species of *Entodinium* is so round as *E. bovis*, and this character is therefore a useful feature for differentiating it from other species of the genus which also are small and devoid of caudal spines (*i.e.* from *E. simplex* Dogiel, *E. dubardi* Buisson forma *dubardi* Dogiel, *E. parvum* Buisson and *E. babići* Wertheim). Its dimensions also serve to distinguish it from other species.

## Differentiation of small non-caudate species of the genus *Entodinium*

Dogiel (1927) remarks upon the difficulty in identifying the unarmed species of *Entodinium*, particularly *E. dubardi dubardi*, *E. simplex* and *E. parvum*. He is uncertain whether, for instance, *E. simplex* and *E. parvum* should be

regarded as a single species, and he only provisionally separates them. He correctly points out a notable similarity between *E. simplex* and *E. dubardi dubardi*. With the material at my disposal I succeeded, however, in defining them and in separating them as two species. None of the existing species are found to be unjustified, but it is necessary to take into consideration some differential characteristics which had previously been disregarded.

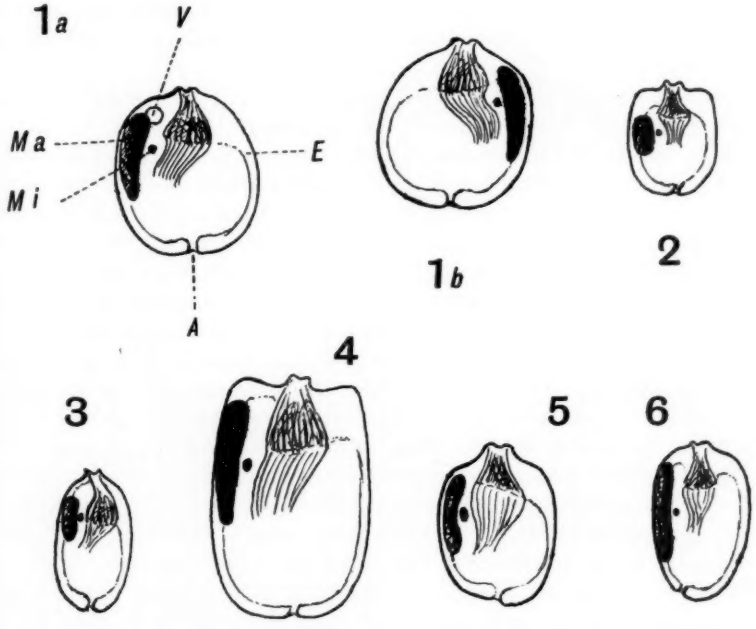


Fig. 1. *Entodinium bovis* n.sp. *a*, right view; *b*, left view. *Ma*, macronucleus; *Mi*, micronucleus; *V*, contractile vacuole; *E*, ectoplasm; *A*, anus. ×900.
Fig. 2. *Entodinium exiguum*. ×800.
Fig. 3. *Entodinium nanellum*. ×1000.
Fig. 4. *Entodinium simplex*. ×950.
Fig. 5. *Entodinium dubardi dubardi*. ×800.
Fig. 6. *Entodinium parvum*. ×800.

Jameson (1925) and Fantham (1920) spoke of *E. bursa* Stein 1859, but Dogiel (1927) has since proved that this species is a complex one and that probably it contained *E. simplex*, *E. ovinum*, *E. dubardi dubardi*, *E. parvum* and *E. vorax*. Evidence for this is the considerable variation in size (55–114 × 37–78 $\mu$) of individuals of the supposed species, whereas true species of *Entodinium* are known to vary little in size. Schuberg's assertion of the ventral position of the contractile vacuole of *E. bursa* is not exact, as it never lies ventrally in this genus.

Gelei (1932) mentions *E. simplex*, giving its length as 30–35 $\mu$, which is incorrect, as Dogiel, who first described it, stated it to be 38–50 $\mu$, and with this my measurements concur. Gelei was probably dealing with *E. dubardi dubardi*.

Jameson (1925) points out "that practically all the specific differences described in this genus are founded on the external contours of the body", and that unless the specific features are strongly marked (*e.g. E. rostratum* or *E. caudatum*) "it is not easy to say where one species begins and another ends". He regrets the lack of attention which has been paid in the past to the internal features.

In Table I are given the main characters of all such species of *Entodinium* which hitherto have not been clearly distinguished.

In *E. exiguum* and *E. nanellum* the differences are well marked in the shape of the macronucleus (see Table I), in the ratio of length to the width of the body and in that of the length of the body to the length of the macronucleus. *E. exiguum* and *E. nanellum* cannot be confused with *E. simplex* because of the obvious difference in size, apart from other distinguishing features. With regard to Dogiel's (1927) reference to the difficulty in distinguishing *E. dubardi dubardi*, *E. simplex* and *E. parvum*, the latter possesses a markedly rounded anal region with the anus considerably displaced dorsally. This can be seen clearly in Buisson's (1924) figures. These two characteristics never appear simultaneously in any other species of the genus, and therefore give a method of defining *E. parvum*, a point which has not been emphasised previously. Buisson's statement that the cuticle of this species is without longitudinal striations is incorrect, for they can be observed clearly after treatment with 10 per cent. iodine alcohol. I have not found exceptionally large individuals such as Buisson has described ($68 \times 36 \mu$), and it is improbable that individuals differing so much in size from the normal could belong to the same species, as the variation in size in species of Entodinia is known to be small. In all probability such larger individuals belonged to *E. babići* which I described from the stomach of *Capra ibex*, or partly to *E. ellipsoideum* from *Bos indicus* (Kofoid and MacLennan, 1930).

*E. simplex* and *E. dubardi dubardi* are to be distinguished by the proportions of the length to breadth of the body and of the length of the macronucleus to that of the body. The position of the anus (in *E. simplex* more ventral, and in *E. dubardi dubardi* more dorsal) and the differences in the thickness of the ectoplasm are also of great value in differentiating these species. In addition *E. simplex* never has an oriment of the preanal lobe, whereas in *E. dubardi dubardi* it is distinctly developed (Figs. 4 and 5). According to Buisson (1924) the position of the contractile vacuole is inconstant, but I agree with Dogiel (1927) that this is incorrect and that it lies (as in all Entodinia) to the left of the anterior end of the macronucleus.

*E. babići* and *E. bovis* n.sp. are distinct species and cannot be confused with the others. *E. babići* (see Table I) is clearly distinguished from *E. simplex*

## Table I

| Species | Author | Host | Dimensions: Length ($L$) $\mu$ | Dimensions: Width ($W$) $\mu$ | Marked features: Shape of macronucleus | Marked features: Position of anus | Ectoplasm | General shape | $L/W$ | $L/l$* |
|---|---|---|---|---|---|---|---|---|---|---|
| *E. exiguum* | Dogiel, 1925 | *Rangifer tarandus*<br>*Bos taurus* | 21–29 | 14–18 | Short and thick | Ventral | Everywhere uniform | Oval, anteriorly level | 1·6 | 3 |
| *E. nanellum* | Dogiel, 1921 | *Aepyceros melampus*<br>*Bos taurus*<br>*Bos indicus*<br>*Ovis aries* | After Dogiel:<br>20–25<br>After Kofoid-MacLennan:<br>22–32<br>After Fantham:<br>25–30 | 10–13<br>12–18<br>— | Elongated and thinner | Ventral | Thicker in the anal region | Elongated, anteriorly more rounded | 2 | 2·75 |
| *E. simplex* | Dogiel, 1925 | *Bos taurus*<br>*Ovis aries*<br>*Capra hircus*<br>*Rangifer tarandus*<br>*Camelus dromedarius* | 38–50 | 21–29 | Elongated, anteriorly thicker | Ventral | Uniform | Elongated, anteriorly level | 1·7–1·74 | 2·2 |
| *E. dubardi dubardi* | Buisson, 1923<br>Dogiel, 1925 | *Capreolus capreolus*<br>*Bubalis cokei*<br>*Aepyceros melampus*<br>*Madoqua* sp.<br>*Gazella thomsoni*<br>*Gazella subgutturosa*<br>*Cervus canadensis lühdorfi*<br>*Tragulus meminna*<br>*Bos taurus*<br>*Ovis aries*<br>*Capra hircus*<br>*Rhaphiceros* sp. | After Buisson:<br>30–40<br>After Dogiel:<br>28–35 | 20–25<br>18–25 | Elongated, in the middle markedly concave, where lies the micronucleus | Dorsal | Thicker in the anal region | Elongated, with an oriment of the ventral lobe | 1·55 | 1·7 |
| *E. parvum* | Buisson, 1923 | *Bubalis lichtensteini*<br>*Cephalophus grimmia*<br>*Limnotragus spekei* | 30–35 | 20–24 | Elongated | Dorsal | Uniform | Elongated, posteriorly more rounded than all other species | 1·55 | 1·7 |
| *E. babiti* | Wertheim, 1933 | *Capra ibex* | 32–53 | 21–39 | Elongated | Dorsal | Uniform | Rounded | 1·42 | 2 |
| *E. bovis* n.sp. | Wertheim, 1934 | *Bos taurus* | 26·25–38·5 | 24·5–31·5 | Elongated, posteriorly thinner | In the middle, sometimes displaced ventrally | Thicker in the anal region | Generally more rounded than all other species | 1·16 | 2 |

* $l$ = length of macronucleus.

(in spite of the similarity in size) by a significant difference in the ratio of length to breadth of body and by the different position of the anus. Between *E. babići* and *E. dubardi dubardi* there is great difference in the relation between the length of the body and the length of the macronucleus. Without emphasising the differences brought out in the table, *E. bovis* is markedly distinguished from all other Entodinia by its exceptional roundness.

## Conclusions

From the data set forth above it is possible to separate a whole series of closely allied species. Thus among the small, non-caudate forms of the genus *Entodinium*, *E. bovis*, *E. babići*, *E. exiguum*, *E. nanellum*, *E. simplex*, *E. dubardi dubardi* and *E. parvum* are distinct and valid species.

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# A DESCRIPTION OF A NECROTIC VIRUS DISEASE AFFECTING TOBACCO AND OTHER PLANTS

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(With Plates V, VI and 2 Text-figures)

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## Introductory

In the glasshouses of the Potato Virus Research Station at Cambridge seedlings of *Nicotiana tabacum* and *N. glutinosa* have frequently been observed which showed a necrosis of one or two of the outside leaves. As a rule in very small seedlings the whole plant was killed, but in older seedlings infection was confined to the outside leaves which became completely necrotic and shrivelled but remained attached to the plant.

The disease appeared regularly in the seed boxes for a number of years and seemed to be most prevalent in the winter and early spring. The same or a similar disease was also observed at the Waite Institute in South Australia during the years 1931–3. For a considerable time it was thought to be of fungal or bacterial origin and little attention was paid to it. Finally inoculations with the sap of infected plants were made on healthy tobacco plants both at Cambridge and at the Waite Institute—the latter by Mr Geoffrey Samuel—and

in both cases necrotic lesions were produced resembling the lesions characteristic of certain virus infections. A suspension from affected seedlings was passed through a bacteria-proof membrane and found to be still infectious, thus confirming the supposition that the disease was caused by a virus. Further study of the virus was undertaken and the results are set forth below.

An interesting point about this disease is the fact that it very rarely becomes systemic in its host; it spreads occasionally to a limited degree in tobacco, and in one case it became partially systemic in *Datura*. The symptoms are almost entirely necrotic, and therefore the name "tobacco necrosis" is suggested for the disease, while if the numerical system of nomenclature advocated by James Johnson is followed the virus itself should be called "Tobacco virus 10".

### Symptomatology and host range

#### *Tobacco (vars. White Burley, Virginia and Vermont)*

Tobacco seedlings in the two-leaf stage when naturally infected collapsed in the seed boxes. Necrosis spread from the base of the stem along the midrib, often killing the seedling within 2 or 3 days of the first appearance of symptoms. Older seedlings, naturally infected, sometimes developed a ring of constricting necrotic tissue around the base of the stem. The necrosis sometimes extended up the midrib of the lowest leaf, causing the death of the leaf; this might occur in succession with several leaves, or alternatively the plant might grow on without evidence of infection except a slight retardation of growth.

When necrosis extended along the midribs and main veins the interstitial green tissue ballooned somewhat, the veins appeared sunken and the leaf curled over a little. Sometimes whitish etched lines appeared on either side of the veins (Pl. V, fig. 3). When collapse occurred it was due to the destruction of the vascular system and not to spread of the necrosis to the interstitial tissues. As already emphasised the general characteristic of infection was the restriction of the symptoms to a few leaves. The virus never became wholly systemic in tobacco in the sense that tobacco mosaic virus becomes systemic, invading every tissue and each new leaf as it expands from the growing point. Sometimes it became partially systemic, especially at high temperatures, in strongly growing plants before the main axis shot up (Pl. V, fig. 3). Necrotic and yellow rings appeared, rarely concentric, and on older and harder leaves they were often several centimetres in diameter and comparatively faint and irregular.

When healthy tobacco plants were inoculated necrotic lesions were produced, often in considerable numbers, on the inoculated leaves (Pl. V, fig. 2). The lesions were usually circular, and in actively growing leaves might later be surrounded by single necrotic rings. Necrosis occasionally spread subsequently along the midrib and veins of one or two of the older leaves.

Symptoms develop in 3–5 days at a mean daily temperature of 15–22° C.

Nicotiana glutinosa

After about 3 days small lesions developed on inoculated leaves. These lesions were very similar in appearance to those produced by viruses of the tobacco mosaic type, but were smaller and did not enlarge much. They rapidly dried out and became white; in this stage the virus content was very low, being finally reduced to nothing. No spread to uninoculated leaves was observed in artificially infected plants of *N. glutinosa*. Natural infections resulted in symptoms similar to those on tobacco, but the spread of the virus was even more restricted.

Datura stramonium

Inoculation to *Datura* produces small circular necrotic lesions beginning as "water soaked" spots. In the first experiment six *Datura* plants were inoculated and all produced the typical small necrotic lesions. In one plant of the six, however, a leaf other than those inoculated showed a whitish ring-like mark near the tip. The necrosis spread very slowly from the tip to the base of the leaf. About 6 weeks later the growing point on the same shoot as the lesion was killed. On one immature leaf near the tip necrosis outlined the vascular system (cf. cowpea, Pl. VI, figs. 3, 4) and the interveinal areas were yellowed. Afterwards within a period of several days all the other growing tips were killed, the mature leaves remained unaffected but the plant grew no further. Some months later symptoms again appeared in one plant of a series on a leaf above the inoculated leaf, but there was no further development.

*Tomato*

In tomato, on sixteen plants inoculated, only local symptoms were produced. Leaflets rubbed with samples of the virus developed necrotic spots after about 5 days, these spread gradually and caused the death of part or all of the leaflet affected. The necrosis did not spread further and the plants remained healthy.

*Potato*

Inoculations were made to twelve young potato plants of the two varieties, President and Arran Victory. No local lesions were formed and there was no subsequent development of infection.

*Cowpea* (Vigna sinensis)

Cowpea is extremely susceptible to infection, but the virus very rarely spreads beyond the inoculated leaf. Lesions develop rapidly and with great regularity, thus making the cowpea a valuable experimental plant. Under optimum conditions the lesions develop in as short a time as 48 hours after inoculation, but the average time is 3–4 days. As they first appear the lesions are circular and pale in colour, but they rapidly turn red and become necrotic. The spread of the virus through the leaf is demonstrated very clearly by the

symptoms. As a lesion enlarges it comes into contact with the veins of the leaf and the necrosis then follows the course of the veins in the manner illustrated in Pl. VI, figs. 3 and 4. Fig. 3 is a photograph of a cowpea leaf about a week after inoculation; fig. 4 is a similar leaf about 9 days after inoculation. At the stage illustrated in fig. 4 abscission usually occurs, but in the event of the leaf remaining attached to the plant there is no further development of infection. The newly formed uninoculated leaves are invariably healthy. In only one case out of many hundred plants inoculated did the virus spread from the inoculated leaf and was recovered from the main stem of the plant. Systemic infection in the cowpea has not been observed under the writers' experimental conditions.

### Distribution of the virus in the infected plant

Some experiments were carried out to ascertain whether the virus was present in the roots of affected tobacco plants. Two types of diseased plants were used, naturally infected seedlings and larger plants artificially inoculated. The experimental procedure was as follows: the root ends were removed and carefully washed, they were then ground up in a mortar and inoculated to one-half of a leaf of healthy tobacco or cowpea. The other half-leaf of the healthy plant was inoculated from the symptomless or infected leaves of the plant under test.

The following is a description of one such experiment. Twelve White Burley tobacco plants, which had themselves been inoculated each on two leaves about 5 weeks earlier, were used as sources of inoculum. After infection these plants had shown about 10–30 lesions per plant. These lesions had spread somewhat on the leaves actually inoculated, but there had been no systemic invasion. Inoculations from the roots and symptomless leaves of the above plants were made. One lesion per plant was produced with the root inoculum in four out of twelve cases and no lesions with the leaf inoculum. Inoculations were repeated from six of the twelve plants, 2 weeks later. One of the six gave two lesions with the root inoculum, but negative results were obtained with the leaf inoculum.

In other cases the numbers of lesions from the root inoculum were a little higher, but the concentration of the virus in the roots appeared nearly always to be low. In the following examples inoculations were made from the roots and from leaves on which symptoms had appeared (Table I).

A few tests were also made to see if the virus was present in parts of infected tobacco leaves other than the actual lesions themselves. Portions of tissue were cut from the following areas: (1) from the midrib of an infected leaf; (2) close to the lesions; (3) far from the lesions. Inoculations with tissue from all three areas to healthy plants gave negative results.

Similarly a test was made to ascertain whether the presence of local lesions of tobacco necrosis on a cowpea leaf would prevent the development of fresh lesions on reinoculation with the same virus. If the virus were systemic in the

leaf, its presence in the cells might be expected to prevent the development of new lesions, by analogy with the work of Thung (1931), Salaman (1933) and Kunkel (1934), who have shown that one virus may protect an infected plant from invasion by a second closely similar virus. In the experiments a number of leaves of cowpea were inoculated. As soon as the lesions had developed, each was ringed with Indian ink and the leaf inoculated again. In every case a fresh set of lesions developed, and this again suggested that the virus was confined to the actual lesion (Pl. VI, fig. 2).

In addition the necrotic virus and the *X* or *Y* virus (Smith, 1931) were inoculated simultaneously on tobacco plants to see if the two would separate out as the second virus became systemic in the host, or whether the presence of

Table I

| Exp. no. | Date | Description of plant | No. of lesions on four test plants produced by inoculation from: Leaves | Roots |
|---|---|---|---|---|
| 1 | 22. ii. 34 | Naturally infected tobacco plant with symptoms on two lower leaves | 20 | 2–3 |
| 2 | 22. ii. 34 | Small tobacco seedling naturally infected | 15–20 | 15–20 |
| 3 | 18. iv. 34 | Small tobacco seedling naturally infected | 12 | 15 |
| 4 | 18. iv. 34 | Large White Burley tobacco plant artificially inoculated, showing local lesions only | 20 | Nil |
| 5 | 21. iv. 34 | Small *N. glutinosa* plant, artificially infected, a single local lesion on inoculated leaf, spreading down vein | Nil | 3 |
| 6 | 21. iv. 34 | As above. Lesion not spreading | Nil | 6 |
| 7 | 21. iv. 34 | Naturally infected White Burley tobacco plant. Symptoms on 3 leaves | 3 | 9 |
| 8 | 21. iv. 34 | Naturally infected White Burley tobacco plant. Only symptom a small dead leaf at base | Nil | 5 |

the second virus might conduce to systemic spread of the necrotic virus. In each experiment the *X* or *Y* virus, as the case might be, became systemic, while the necrotic virus remained in the inoculated leaf. Pl. V, fig. 4 illustrates one such experiment. On the two lower leaves may be seen the two types of lesions characteristic of the necrotic virus and virus *X*, while the other leaves show symptoms of the *X* virus alone.

A further experiment was carried out to ascertain whether the presence of virus *X* in a systemically infected plant would inhibit the entrance of the tobacco necrosis virus. Six plants of *Nicotiana langsdorffii* showing systemic infection with *X* were inoculated with the virus of tobacco necrosis. All six developed the characteristic lesions of this virus, actually on top of the rings caused by virus *X*.

## PHYSICAL PROPERTIES

### (1) *Dilution end-point*

The lesions of tobacco necrosis on tobacco and cowpea were sufficiently distinct for counting, and the possibility of using them to estimate virus concentration was tested by some preliminary dilution experiments. It has been shown (Holmes, 1929; Samuel and Bald, 1933) that the relation between the

number of lesions and dilution of ordinary tobacco mosaic virus is not linear: similarly with the more concentrated samples of tobacco necrosis, dilution decreased the numbers of lesions in less than linear proportion. In one experiment the virus used was diluted with water weakly buffered with a phosphate-phthallate mixture at *p*H 6·3–6·5. The mean numbers of lesions produced on twenty replicates (half-leaves of tobacco) were as follows:

| Dilution | 1 : 2 | 1 : 4 | 1 : 8 | 1 : 16 | 1 : 32 |
|---|---|---|---|---|---|
| Lesions per half-leaf | 27·0 | 25·6 | 21·1 | 19·7 | 12·7 |

The experiment was arranged on a randomised plan with the 1 : 2, 1 : 4, 1 : 16 and 1 : 32 dilutions inoculated in every case on opposite half-leaves against the 1 : 8. The standard error of the difference between the 1 : 8 dilution and any other was 1·38 lesions, and between any two of the remainder 1·95 lesions, so that although dilution significantly decreased the number of lesions produced, the decrease was much less than would be expected. In another experiment the following results were obtained:

| Dilution | 1 : 20 | 1 : 60 | 1 : 180 | 1 : 540 |
|---|---|---|---|---|
| Lesions per half-leaf | 23·1 | 21·9 | 15·7 | 6·2 |

At the lower concentrations the number of lesions tended to approach a linear relation with the dilution, as may be seen clearly by comparing the ratios of lesions for successive concentrations with the dilution ratios in the second experiment. The former were 1 : 1·1, 1 : 1·4, and 1 : 2·5; the latter was in every case 3. Whether at lower concentrations a linear relation might be obtained, and under what conditions, remain to be determined. There is evidence that the *p*H, the amounts of plant debris, and the concentration of buffer salts in the inoculum affect the numbers of lesions produced. There is, however, no doubt that differences in concentration of the tobacco necrosis virus can be determined by the primary lesion method, provided that the virus is suspended in comparable media.

The extent to which a sample of virus may be diluted without entirely losing its virulence depends on its initial concentration. The end-point for such material as that used in the first dilution experiment—tobacco leaves covered with primary lesions—was estimated to be about 1 : 2000. When the virus was extracted from cowpea leaves covered with lesions as in the second experiment, it was somewhat higher, of the order of 1 : 10,000. A sand and paper pulp filtrate of this material was infectious to a dilution of about 1 : 2000.

### (2) *Longevity* in vitro

Two experiments were carried out to determine roughly the resistance of the virus to ageing in extracted sap. In the first experiment, samples of juice from tobacco leaves covered with local lesions were held at temperatures fluctuating within one or two degrees of 2 and 29° C. The sample held at 29° C. was found to be active at 4 hours and at 4 days at a dilution of 1 : 30, slightly active at 9 days and not viable at 40 days. After 9 days the sample held at 2° C. produced 60 lesions on eleven half-leaves of tobacco compared with 7

lesions obtained from the sample held at 29° C. At 40 days there were either one or two lesions on nine out of fifteen half-leaves inoculated with a 1 : 5 dilution. The other experiment was arranged so that counts of lesions from successive inoculations would be comparable. A sample of inoculum was held at a temperature fluctuating about a mean of 21° C. Inoculations were made to ten half-leaves of White Burley tobacco. The average numbers of lesions produced on a leaf were as follows: zero, 48 lesions; 1 hour, 50 lesions; 1 day, 34 lesions; 2 days, 24 lesions; 4 days, 7·9 lesions. If the numbers of lesions were reduced continuously at the same rate, the sample of virus would have lost its virulence completely in about 20 days. This result agrees fairly well with those obtained from the other experiment.

### (3) *Thermal death-point*

The temperature at which the necrotic virus becomes inactivated was tested by the following method. Undiluted virus sap from tobacco or cowpea was held in 2 c.c. fractions in closed tubes, completely immersed in water for

Table II. *Thermal death-point of the necrotic virus.*

| No. of tests | Temp. ° C. | No. of plants infected of four inoculated | | Approximate no. of local lesions per plant | |
|---|---|---|---|---|---|
| | | Control | Exp. | Control | Exp. |
| 1 | 50 | 4 | 4 | 20–30 | 20 |
| 1 | 55 | 4 | 4 | 20–30 | 10 |
| 2 | 60 | 8 | 6 | 20–30 | 4–6 |
| 2 | 65 | 4 | 4 | 20–30 | 3 |
| 2 | 66 | 8 | 4 | 10–30 | 1–2 |
| 2 | 67 | 8 | 2 | 20–30 | 1–5 |
| 2 | 68 | 8 | 2 | 20–30 | 2–4 |
| 2 | 69 | 4 | 1 | 20–30 | 2 |
| 2 | 70 | 8 | 5 | 20–30 | 2 |
| 1 | 71 | 4 | 1 | 20–30 | 2 |
| 1 | 72 | 4 | 0 | 20–30 | 0 |
| 1 | 73 | 4 | 0 | 20–30 | 0 |
| 1 | 74 | 4 | 0 | 20–30 | 0 |

Experimental plants inoculated with the heated virus sap, controls with the same virus sap unheated.

10 min. periods. The sap was first clarified of all visible particles by passage of a sand and pulp filter. Cowpeas were used throughout as test plants. The results of the tests on thermal death-point are shown in Table II. It will be seen from the data given in the table that the virus ceases to be viable after exposure to temperatures between 70 and 72° C., but judging from the numbers of primary lesions even a temperature of 55° C. for 10 min. reduces the virulence to some extent.

### (4) *Resistance to alcohol*

The virus of tobacco necrosis is unusually resistant to the action of alcohol. Tests were made with various concentrations from 50 to 99 per cent. for periods of 2–71 hours. Absolute alcohol was used in each test and diluted down to the appropriate percentage with virus sap, used undiluted but cleared by passage through a sand and pulp filter. All inoculations were made to cowpea.

It was found that positive results were obtained with the virus even after 71 hours in 99 per cent. alcohol. In the first two experiments at this concentration, the ordinary laboratory supply of absolute alcohol was used. For the remainder of the tests a special supply was obtained which had been dried over quicklime and redistilled. In all experiments with 99 per cent. alcohol control tests with the virus at dilutions of 1 : 100 of water were also made. The results are given in Table III.

In the last two columns are given the numbers of lesions counted or estimated on the test plants. Those for 3-hour periods have been used for the construction of Text-fig. 1 which gives a roughly quantitative idea of the effect of

Table III. *Resistance of the tobacco necrosis virus to alcohol.*

| Exp. no. | Alcohol per-centage | Time in alcohol (hours) | Plants infected out of six inoculated | | No. of lesions per plant (approximate) | |
|---|---|---|---|---|---|---|
| | | | Control | Exp. | Control | Exp. |
| 1 | 50 | 3 | 6 | 6 | 40–50 | 40–50 |
| 2 | 60 | 3 | 6 | 6 | 40–50 | 20–30 |
| 3 | 60 | 3 | 6 | 6 | 40–50 | 20 |
| 4 | 70 | 3 | 6 | 6 | 40–50 | 10–15 |
| 5 | 70 | 3 | 6 | 6 | 40–50 | 10 |
| 6 | 70 | 3 | 6 | 6 | 40–50 | 20 |
| 7 | 80 | 3 | 6 | 6 | 40–50 | 20 |
| 8 | 80 | 3 | 6 | 4 | 40–50 | 4 |
| 9 | 80 | 3 | 6 | 6 | 40–50 | 10 |
| 10*a* | 90 | 3 | 6 | 2 | 40–50 | 4 |
| 10*b* | 90 | 3 | 6 | 4 | 40–50 | 5 |
| 11 | 90 | 6 | 6 | 1 | 10–15 | 2 |
| 12 | 95 | 6 | 6 | 1 | 40–50 | 2 |
| 13 | 99 | 2 | 6 | 6 | 40–50 | 10–12 |
| 14 | 99 | 4½ | 5 | 5 | 40–50 | 10–12 |
| 15 | 99 | 6 | 6 | 1 | 40–50 | 3 |
| 16*a* | 99 | 24 | 4 | 2 | 10–15 | 2 |
| 16*b* | 99 | 24 | 6 | 6 | 40–50 | 5–10 |
| 16*c* | 99 | 24 | 6 | 4 | 40–50 | 5–10 |
| 17*a* | 99 | 48 | 6 | 4 | 40–50 | 2 |
| 17*b* | 99 | 48 | 6 | 4 | 40–50 | 2 |
| 18 | 99 | 71 | 6 | 3 | 40–50 | 2 |

Experimental plants inoculated with the virus sap-alcohol mixtures, control plants with the same virus sap untouched. In the experiments with 99 per cent. alcohol additional controls were inoculated with 1 : 100 virus sap in water.

The leaves of all inoculated plants were washed in running water immediately after inoculation.

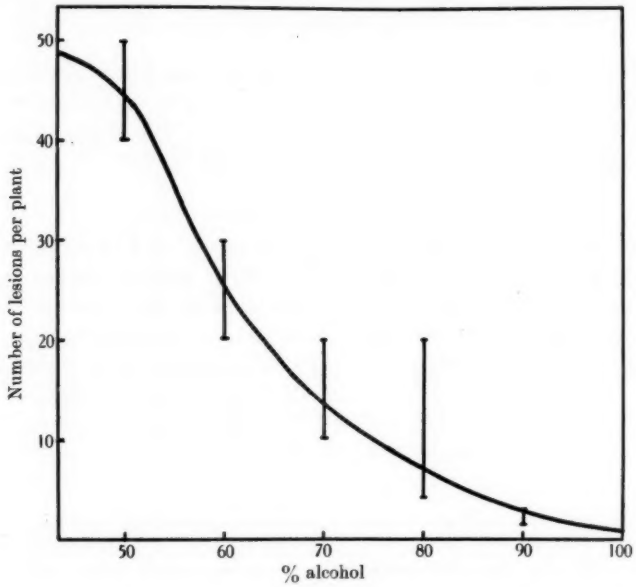
different concentrations of alcohol on the virus. The vertical lines at each temperature represent lesions on a single plant and cover the range of variation found in different experiments. A freehand curve is drawn through them to suggest a continuous fall in virulence with increasing concentrations of alcohol above 50 per cent.

## Transmission studies

### *By seed*

Infection was frequently found in White Burley tobacco within a few weeks of germination. Minute plants, with only the first two or three seedling leaves expanded, became necrotic and were killed, perhaps one or two or even

a dozen in a box of 200. This suggested the possibility of seed transmission, although the soil of the seed boxes was unsterilised and the conditions were such that insect transmission could not be excluded. In June 1934 ten seed capsules were taken, five from the ordinary stock of White Burley in which sporadic infections occurred, and five from an inoculated plant in which the disease had become partly systemic. About 200 seeds from each were sown in a Petri dish on filter-paper moistened with culture solution. The seeds germinated and the seedlings grew without symptoms of infection, but they had not put out the third leaf when growth almost ceased owing to the overcrowded conditions. The seedlings were crushed with a little distilled water and inoculated




Text-fig. 1

to tobacco, but no symptoms were produced on the test plants. The evidence for seed transmission so far as it went was negative, but the question must remain open until more elaborate experiments are possible.

*By spraying*

Owing to the fact that the virus of tobacco necrosis appears to spread among tobacco plants in the glasshouse not only in the absence of vectors but even when the plants are grown in sterilised soil, the following experiment was performed. Seed of White Burley tobacco was sown in boxes of sand, both boxes and sand being sterilised, and the seedlings were watered with a nutrient solution every 2 or 3 days. The disease made its appearance when the seedlings were about half to three-quarters of an inch across, 42 days after the seed was

sown. It appeared simultaneously in boxes of similar tobacco seedlings grown in unsterilised soil in the same glasshouse.

Some experiments to investigate the possibility that the virus is air-borne were then instigated and are still in progress. It may be of interest, however, to describe the following experiment. A sand and pulp filtrate of the tobacco necrosis virus was sprayed on to healthy cowpeas by means of an atomiser. Out of a total of twenty-four plants sprayed twelve developed the local lesions characteristic of this virus; the lesions were not numerous, averaging two or three per leaf. This result is in accordance with the findings of Duggar and Johnson (1933), who infected tobacco plants by spraying them with a suspension of tobacco mosaic virus.

*By insects, etc.*

A series of experiments was performed to discover if any of the following insects, etc., could transmit the virus of tobacco necrosis: *Myzus persicae* Sulz., *Thrips tabaci*, and the red spider *Tetranychus telarius*. These three were selected as being the commonest potential vectors in glasshouses.

Myzus persicae *Sulz.*

Twelve tests were carried out with this aphis, but all gave negative results. In six the insects were first colonised on White Burley tobacco seedlings, affected on one or two leaves with tobacco necrosis, and then transferred to healthy seedlings. In the other six the aphides were colonised first upon White Burley plants affected with a virus complex of potato virus *Y* and tobacco necrosis. In these latter tests the insects selected out the virus *Y* leaving behind the necrotic virus. This selective transmission at least proved that the insects had actually fed on the infected plants.

Thrips tabaci

Six tests carried out with larval thrips of this species have given negative results, but further tests are necessary.

*Red spider* (Tetranychus telarius)

A fairly extensive series of tests was made of the red spider as a possible vector. In a number of cases lesions, somewhat similar in appearance to those of tobacco necrosis, developed on the leaves of White Burley tobacco colonised with red spider from infected plants, but it was found impossible to produce infection in healthy plants by mechanical inoculation from the affected tissue. The writers formed the conclusion therefore that the lesions were due to the feeding of the red spider and had no connection with the virus.

The vector of this virus, if any such exists, is therefore at present unknown.

FILTRATION THROUGH GRADED COLLODION MEMBRANES

Of recent years a technique for producing collodion membranes of graded and uniform pore size has been developed (Elford, 1931). The membranes are

called "Gradocol" membranes, and if correctly prepared they allow the particle size of viruses to be calculated with considerable accuracy. Since a comprehensive study of the filtration of plant viruses through these membranes is now in progress and will be published shortly it is not proposed to give here a description of the method. The reader is referred to various papers by Elford and his colleagues (Elford and Andrewes, 1932), whose methods have been closely followed in these studies.

The virus sap was extracted from infected cowpeas and used undiluted. It was cleared of all visible particles and prepared for filtration by passage

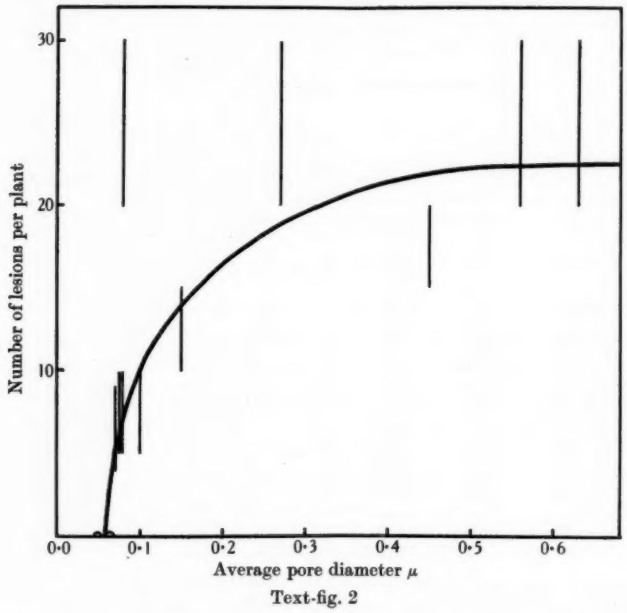
Table IV. *Filtration of the virus of tobacco necrosis through graded collodion membranes.*

| pH | Titre of sand and pulp filtrate | Membrane thickness mm. | Av. pore diam. of membrane $\mu$ | Controls | | Exp. plants | | Approx. no. of lesions per plant: Controls (sand and pulp filtrate undiluted) | Approx. no. of lesions per plant: Exp. plants |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Inoc. | Infected | Inoc. | Infected | | |
| 6·6 | Not tested | 0·20 | 0·63 | 3 | 3 | 6 | 6 | 20–30 | 20–30 |
| 6·6 | " " | 0·18 | 0·56 | 3 | 3 | 6 | 6 | 20–30 | 20–30 |
| 6·6 | " " | 0·20 | 0·45 | 4 | 4 | 3 | 3 | 20–30 | 15–20 |
| 6·4 | 1 : 100 | 0·20 | 0·35 | 5 | 5 | 5 | 4 | 20–30 | 5–10 |
| 6·8 | 1 : 1000 | 0·20 | 0·27 | 6 | 6 | 6 | 6 | 20–30 | 20–30 |
| 6·2 | 1 : 1000 | 0·26 | 0·26 | 5 | 5 | 6 | 6 | 20–30 | 5–10 |
| 6·4 | 1 : 1000 | 0·194 | 0·15 | 3 | 3 | 6 | 5 | 20–30 | 10–15 |
| 6·0 | 1 : 100 | 0·195 | 0·10 | 3 | 3 | 5 | 5 | 20–30 | 10–15 |
| 6·8 | 1 : 1000 | 0·20 | 0·10 | 5 | 5 | 5 | 5 | 20–30 | 5–10 |
| 6·8 | Less than 1 : 100 | 0·21 | 0·082 | 4 | 4 | 5 | 1 | 10–20 | 1 |
| 6·8 | 1 : 1000 | 0·21 | 0·080 | 4 | 4 | 6 | 6 | 20–30 | 20–30 |
| 6·3 | 1 : 1000 | 0·22 | 0·078 | 4 | 4 | 7 | 7 | 20–30 | 5–10 |
| 6·6 | 1 : 1000 | 0·22 | 0·074 | 6 | 6 | 7 | 7 | 20–30 | 5–10 |
| 6·8 | 1 : 1000 | 0·20 | 0·070 | 3 | 3 | 7 | 6 | 20–30 | 5–10 |
| 6·8 | 1 : 1000 | 0·23 | 0·060 | 3 | 3 | 6 | 0 | 20–30 | — |
| 6·4 | 1 : 1000 | 0·23 | 0·054 | 4 | 4 | 8 | 0 | 20–30 | — |
| 6·6 | 1 : 1000 | 0·23 | 0·054 | 4 | 4 | 6 | 0 | 20–30 | — |

The sources of virus sap used throughout were infected cowpea leaves; the filtration pressure was 34 lb. per square inch, and the amount filtered in each case was 5 c.c. Inoculations were made to cowpeas. Time of flow of water values were taken for each disc before filtration.

through a sand and pulp filter (Barnard and Elford, 1931). Cowpeas were used throughout as test plants. Owing to the variation in virus content of the infected cowpea leaves used as sources of virus for filtration, control inoculations were made at dilutions of 1 : 100 and 1 : 1000 to test the titre of the virus suspension. It was found that in suspensions of low virus content the virus failed to pass a membrane of an average pore diameter that normally would allow its passage, especially if the membrane were of slightly greater thickness than usual. The results of the ultra-filtration experiments are given in Table IV and in Text-fig. 2. It will be seen that the filtration end-point is 0·06$\mu$, which by Elford's method of calculation (Elford, 1933) gives an average particle diameter of 20–30$\mu\mu$. The virus of tobacco necrosis therefore appears to be one of the smallest of the plant viruses. It is probably as small as, if not smaller than, the virus of tobacco mosaic (Tobacco virus 1).

The figures for the number of plants infected show a very sharp end-point, *i.e.* passage of the virus through the membrane ceased suddenly with decrease of average pore diameter. As a check on these figures rough estimates of the numbers of lesions produced by undiluted juice filtered through sand and pulp and through collodion membranes were made, and as mentioned above dilutions of 1 : 100 and 1 : 1000 were tested for the presence or absence of the virus to make sure that initially the juice was fairly concentrated. It will be seen from the nature of the figures that no distinction would appear between a virus




Text-fig. 2

suspension just sufficiently concentrated to infect a plant at 1 : 1000 dilution and one strong enough to infect at 1 : 10,000, and that according to the results of the dilution experiments (p. 236) the numbers of lesions given by a concentrated sample might not be greatly reduced by reducing its virus content to one-tenth. Bearing these facts in mind the numbers of lesions in the last column of Table IV were plotted against the average pore diameter (Text-fig. 2), only those for the samples virulent at 1 : 1000 dilution and the first untested samples being used. If it were assumed that the two samples at average pore diameter 0·08$\mu$ and 0·27$\mu$ were initially more concentrated than the others, and that the numbers of lesions were not reduced even if passage through the filter reduced the numbers of virus particles, the lower values would represent the effect of decreasing average pore diameter on the concentration of the filtered virus. This, the less favourable interpretation of the data, indicates a reduction of virulence by filtration over a considerable range

of average pore diameter, but the end-point is still very clearly marked. The method gives consistent and reproducible results provided the virus sample to be filtered is strong enough to make allowance for some degree of retention or adsorption by the filter.

### COMPARISON OF THE VIRUS OF TOBACCO NECROSIS WITH THAT OF TOBACCO RINGSPOT

In some respects the symptoms induced by the virus of tobacco necrosis resemble those of the tobacco ringspot virus described by Wingard (1928) and others. The two viruses have been carefully compared at Cambridge and the following facts have been compiled to aid differentiation of the two.

Firstly with regard to the symptom picture on cowpea and White Burley tobacco, while the local lesions produced by the two viruses on cowpea are closely similar, further developments suffice to distinguish between them. The ringspot virus is always systemic and usually fatal to the cowpea, the necrotic virus rarely if ever progresses beyond the inoculated leaves. Similarly on tobacco, the ringspot virus always becomes systemic, that of tobacco necrosis rarely spreads to other leaves and never becomes completely systemic. In addition the concentric rings produced by the former virus on tobacco are very characteristic. As regards the physical properties of the two viruses, the thermal death-point of tobacco necrosis is about 72° C., while that of tobacco ringspot has been found to lie between 60 and 65° C.

In their power of resistance to alcohol they are very different, and this fact alone is sufficient to distinguish between the two. Tobacco ringspot is inactivated by 24 hours' exposure to 60 per cent. alcohol, while the necrotic virus withstands 24 hours' exposure to 99 per cent. alcohol.

The particle size of the virus of tobacco necrosis has been found, by the method of ultra-filtration, to be 20–30 $\mu\mu$, while that of tobacco ringspot is 75–120 $\mu\mu$. The last figure is only an approximate value.

### DISCUSSION

The virus of tobacco necrosis is unusual in several respects. Firstly it tends to remain localised in its host plant and hardly ever becomes normally systemic above ground, but it may occasionally be found in the roots of plants in which symptoms are confined to the inoculated leaves.

No positive evidence on the natural dissemination of the disease has been obtained, although transmission experiments have not been very extensive. The question of transmission may be bound up with the peculiar distribution of the virus in the plant; for instance, an insect in order to pick up the virus would have to feed either on the roots or on comparatively restricted areas of leaf tissue, which areas are generally necrotic or adjacent to necroses.

It may, however, be of significance that healthy cowpeas can be infected

with tobacco necrosis by spraying them with a suspension of the virus from an atomiser.

Seed transmission would imply the passage of the virus to the developing seed, although it does not normally pass to the growing point. Some of the *in vitro* properties of tobacco necrosis resemble those of tobacco mosaic, notably its small particle size and its resistance to alcohol; but others are very different, for instance, its rapid inactivation at ordinary temperatures and its lower thermal death-point.

Tobacco necrosis is clearly distinct from the American tobacco ringspot. It seems to have some points in common with a ringspot virus described by Böhme (1933) in Germany, but the fact that Böhme's virus becomes systemic in potato (p. 233) and in other host plants seems to differentiate the two.

## Summary

An account is given of an apparently undescribed virus disease. The virus appears spontaneously in the glasshouse affecting tobacco seedlings in the seed boxes. The symptoms on a number of hosts are described. The chief characteristic of the virus is its failure to become systemic in any host plant so far tested. It is, however, occasionally found in the roots of young tobacco plants, usually in the case of those naturally infected. Certain physical properties of the virus have been investigated. Its dilution end-point appears to be about 1 : 10,000; its longevity in extracted sap is about 20 days, the thermal death-point is 72° C. The virus shows a strong resistance to alcohol, remaining viable in 99 per cent. alcohol for 71 hours so far as tested.

It is not known how the virus is transmitted in nature, and no insect vector has as yet been identified.

The virus has been transmitted to healthy cowpeas by spraying them with a suspension of the virus by means of an atomiser.

By the Elford method of ultra-filtration the particle size is found to be 20–30 $\mu\mu$.

The virus is compared with that of the American tobacco ringspot, and methods of differentiating the two are indicated.

## Acknowledgments

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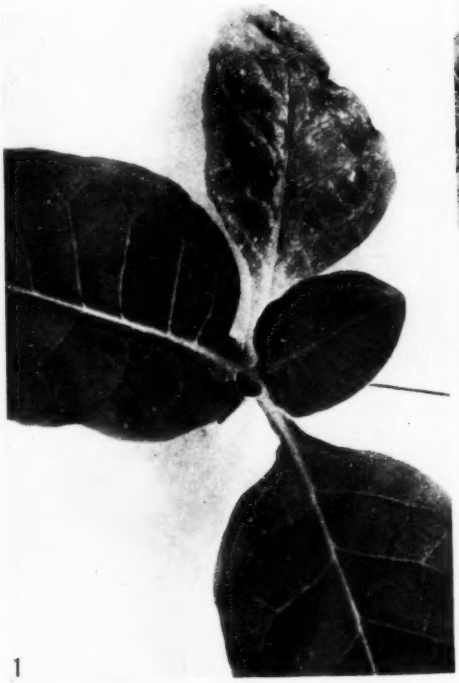

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## EXPLANATION OF PLATES V, VI

### PLATE V

Fig. 1. Seedling of White Burley tobacco naturally infected with the virus of tobacco necrosis.

Fig. 2. Seedling of White Burley tobacco artificially inoculated; note the lesions confined to the inoculated leaves.

Fig. 3. Young plant of White Burley tobacco showing a partially systemic invasion by the virus.

Fig. 4. Young plant of White Burley tobacco inoculated with a mixture of the virus of tobacco necrosis and potato virus *X*. Note the lesions produced by the two viruses on the two inoculated outside leaves. The three inner leaves show systemic symptoms of the *X* virus alone.

### PLATE VI

Fig. 1. Two seedlings of cowpea inoculated with the viruses of tobacco necrosis and tobacco ringspot respectively. The upper plant shows the lesions produced by the tobacco necrosis, the lower shows the lesions characteristic of tobacco ringspot. Note that in the upper plant the new leaves are healthy, while in the lower the new leaves already show puckering due to systemic invasion by the ringspot virus.

Fig. 2. Leaf of cowpea which has been twice inoculated with the virus of tobacco necrosis. The lesions caused by the first inoculation are ringed with Indian ink. The development of a second group of lesions close to the first group suggests that there is no virus in the leaf outside the necrotic spots.

Figs. 3 and 4. Two leaves of cowpea, 7 and 9 days respectively after inoculation with the virus of tobacco necrosis. Note the necrosis is following the veins of the leaves.

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# OBSERVATIONS UPON A "*TRICHOMONAS*" FROM POND WATER

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(With Plate VII, containing Figs. 1–11)

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## I. Introduction

On July 25th, 1934, Prof. Keilin handed me a small tube, which he had received two days previously, containing water and the centrifuged sediment from a pond in Lincolnshire. The water was pink owing to the presence of large numbers of a pink bacillus (unidentified). In the sediment were many Protozoa, including *Paramecium caudatum*, *Cyclidium* sp., *Difflugia* sp., some small amoebae, *Euglena viridis*, *Astasia* sp., a species of *Hexamita* and some small flagellates which were not identified. But the organism which was most numerous was a small flagellate which had three anterior flagella and a short undulating membrane at the anterior end of the body. The presence in pond water of a flagellate bearing such a striking resemblance to the parasitic flagellate *Trichomonas* seemed to be a matter worth investigating. The tube was kept upon the bench (18–22° C.) for a fortnight. The trichomonad-like flagellate multiplied rapidly for the first few days and then slowly died out.

On July 30th approximately half a litre of water from the same pond was received. No trichomonad-like flagellates were found in the water, or in the mud on sedimentation. The whole volume was centrifuged in clean containers at 2000 revolutions per minute for 10 min. The sediment, with a little of the supernatant fluid, was put into three sterile test-tubes, each tube being filled to the depth of about 3 in. The supernatant fluid was stored in two sterile flasks. Extensive examination of the sediment failed to reveal any trichomonad-like flagellates, but 7 days later they were found in all three tubes, but

not in the flasks. Dividing forms were seen and they became numerous. Other Protozoa, similar to those in the first specimen, were also present.

On September 5th, 1934, I visited the pond. It was situated in Lincolnshire about 3 miles from the shores of the Wash, and lay in a pasture field about 10 yd. from a secondary public road. It was quite small. As 1934 was a hot, dry summer the pond was very concentrated, and about 2 ft. of baked mud was exposed above the level of the water. The surface of the water was covered with *Lemna*, and a sour smell of decay was noticeable when the water was stirred. The bottom of the pond consisted of thick black mud and decaying leaves, for a group of trees (Dutch elm, fir, sycamore, horse-chestnut, and lime) stood about 5 yd. from the edge of the pond. A cow was grazing in the field. A farm, with cowsheds, stables, and manure heaps, was situated on the opposite side of the road and no sign of direct drainage from the farm to the pond was visible. About 5 litres of water and mud were collected in clean bottles. On sedimentation no trichomonad-like flagellates were found. The whole volume of water and mud was centrifuged at 2000 revolutions per minute for 10 min., but examination of the sediment failed to reveal the flagellates. Other Protozoa, similar to those found in the previous specimens, were present. The sediment, with a little of the supernatant fluid, was put into six sterile tubes and one large jar. Three weeks later many of the trichomonad-like flagellates were found in the mud in the large jar and in one test-tube, but none were found in the supernatant fluid. Many stages of division were seen.

## II. METHODS OF CULTIVATION AT ROOM TEMPERATURE (17–24° C.)

Cultures were made by inoculating sediment rich in the trichomonad-like flagellates into sterile test-tubes each of which contained two boiled wheat grains and pond, or rain, water which had been sterilised by boiling, or by passage through a Seitz filter. The flagellates multiplied in this medium and lived for about a month. Subcultures at first grew well, but *Paramecium*, *Astasia*, and *Hexamita*, and later *Bodo*, were present and the two latter forms multiplied more rapidly than the trichomonad-like flagellate and gradually swamped it. Therefore after 2 months this method was abandoned. Attempts to grow the flagellate using Cambridge tap water failed; but this was not surprising, as failure occurred years ago when attempts were made to cultivate *Spirostomum ambiguum* in it by the wheat grain method, whereas flourishing cultures of this ciliate were obtained when Manchester tap water, which is free from chalk, was used.

Hay infusion was prepared by adding 10 g. of chopped hay to a flask containing half a litre of tap water. The flask was plugged and the infusion was steamed on three consecutive days. The infusion was not used until it had stood at room temperature for 3 weeks to a month, as newly made infusions were found unsuitable as a culture medium. Test-tubes containing 5 c.c. of the hay infusion were used. Heavy growths of the trichomonad-like flagellate appeared after 5–10 days and the cultures lasted from 6 to 8 weeks. A strain

was isolated on 10. viii. 34 from the specimen of pond water received on July 30th and is still (8. ii. 35) growing luxuriantly, being in its 12th generation. The initial cultures contained *Astasia* and *Hexamita*, but these died out in the second subculture, leaving a "pure" culture of the trichomonad-like flagellate. By "pure" is meant a culture containing only the one species of protozoan; a mixed flora of bacteria is always present. In the media described below these bacteria were originally inoculated, together with the protozoan, from the pond water, for the media were sterile and all precautions against contamination were taken. But the hay infusions used were not always sterile bacteriologically. Hay infusions in which a growth of bacteria had occurred produced as rich cultures of the trichomonad-like flagellate as a sterile infusion of the same age. Though bacteria might be present in these hay infusions before inoculation Protozoa were not, for the steaming on three successive days adequately prevented this.

Excellent cultures were obtained in tubes containing 5 c.c. of a medium consisting of 1 part of inactivated horse-serum to 9 parts of 0·5 per cent. salt solution. A little solid, sterile rice starch was added to each tube before inoculation. A strain isolated on 10. viii. 34, from the same source as the strain grown in hay infusion described above, is still growing luxuriantly and is in its 12th generation. The flagellates become plentiful about the 7th–14th day and the subcultures last from 5 to 11 weeks. *Hexamita* was present in the initial culture and the first subculture, but it died out in the latter before the trichomonad-like flagellate and a "pure" strain was obtained.

A medium consisting of an inspissated horse-serum slope covered with the above-described liquid-serum medium gave very rich cultures either with or without starch. In this medium growth was very rapid and thick cultures were obtained by the 3rd to 5th day. They died out in 3–4 weeks. No growth was obtained when an inspissated whole-egg slope was substituted for the serum slope, though the covering liquid medium remained the same. The flagellates grew thickly upon an inspissated horse-serum slope covered with inactivated horse serum 1:8 in Ringer's fluid (Dobell and Laidlaw, 1926). Strains could be changed from the serum media to hay infusion and from the latter to the former without any ill effects.

### III. The effect of temperature upon the growth of the flagellate in culture

The trichomonad-like flagellate grew equally well in the fluctuating temperature of the laboratory (17–24° C.) and in an incubator running at a constant temperature of 23° C. Cultures grown at 11° C. (in hay infusion, the liquid-serum medium with starch, and on the inspissated horse-serum slopes covered with the liquid-serum medium with or without starch) were as rich as those grown at 17–24° C., but multiplication was less rapid and the cultures lasted slightly longer.

At +4° C. cultures of the flagellate survived over a month, but no multiplication occurred.

At 31° C. excellent cultures were obtained in all the above media. Growth was much more rapid than at 17–24° C., but the cultures died out sooner, few surviving for more than a month. Strains have been kept at this temperature since 29. x. 34 and still give luxuriant growths.

Repeated attempts to grow the flagellates at 37° C. have failed, all individuals dying out within 48 hours. This applies to subcultures made, from time to time, from the strains kept at 31° C. as well as to those made from strains grown at room temperature, and to strains grown in all the types of media described above.

No difference in size or general appearance was noticeable in strains grown at the different temperatures, with the exception of those kept at +4° C. These flagellates became almost twice the normal size, probably because at this temperature, though division is suspended, feeding is not.

## IV. Cytological methods

Lugol's iodine was used in counting the flagella. Flagellates grown by any of the above methods were extremely difficult to fix and stain well, but the best preparations were made by fixing for 2 hours in Bouin's fixative and staining with Heidenhain's iron haematoxylin. Other fixatives were used for comparison, the best being Schaudinn's fluid. Flagellates grown in the serum media fixed more naturally than those grown in hay infusion.

## V. Description of the flagellate

Observers well acquainted with *Trichomonas*, both in faeces and in culture, become familiar with its characteristic movement. The movement of this flagellate from the pond is similar. Living specimens measure 7–11 × 3–6 $\mu$, though rounded forms may measure only 5–7 $\mu$. The average individual is more rounded than *Trichomonas hominis*, and from the middle of the posterior end of such forms a spike projects which varies in length. The flagellates are extraordinarily varied in form and very metabolic. A long acquaintance with *T. hominis*, *Trichomonas* sp. from *Macacus rhesus* and *M. nemestrinus*, and with *Trichomonas batrachorum* and *T. sanguisugae*, in culture, as well as with many trichomonads in the faeces of their hosts, has never revealed to me such plasticity of form as is found in these flagellates from the pond. Whereas in some cultures they were almost globular, in others they bore a striking resemblance to *T. hominis*, whilst in others the majority had the carrot-like shape of *Bodo caudatus*, and in yet others they were extremely elongated and narrow, with a greatly reduced undulating membrane. The preponderance of any one type seems to be correlated, to some extent, with the age of the culture. Young cultures tend to contain a greater proportion of the globular forms, whilst in older cultures more elongated types are most numerous. But

by careful search of a culture at any age all the different shapes can be found. The nature of the medium did not appear to have any influence on the form. On fixation some shrinkage occurs, and the variety of form is much less noticeable than in the living culture.

Three free flagella arise together from the anterior end, and this number seems to be invariable. They are of unequal length, the longest being about once-and-a-half to twice the length of the body, and the shortest about half the length of the longest. The third is a little shorter than the longest. From the point of insertion of the three anterior flagella an undulating membrane arises. In the spherical or the typical "trichomonad" forms it extends about half-way towards the posterior end, but in the more elongated forms it is very short. The flagellum bordering it does not project freely beyond its termination. A well-developed mouth is visible at the anterior end, in the normal position for that organ in trichomonads. The flagellate feeds voraciously upon the bacteria present in the cultures, and in media containing starch it gorges itself with that too. Numerous food vacuoles are present. The nucleus, which lies at the anterior end, below the point of insertion of the anterior flagella, is to be distinguished from the surrounding cytoplasm by its greater density. An axostyle is not visible in the living flagellate.

In fixed and stained preparations the flagella are seen to arise from a well-developed blepharoplast. (The same cytological terminology is used here as previously; see Bishop, 1931.) The flagellum bordering the undulating membrane is extremely thin and sometimes very difficult to distinguish. In some forms (Fig. 2) the undulating membrane is very short, whereas in others (Figs. 3 and 5) it ends near the middle of the body. A free projection of the bordering flagellum, such as occurs in most trichomonads, has never been seen in the many preparations studied. The basal fibre, which in all known trichomonads lies below the undulating membrane, is always absent in this organism. The spherical or oval nucleus has a delicate nuclear membrane. In the resting stage a single round karyosome lies centrally or sub-centrally, and occasionally (Fig. 2) two are seen. In some individuals (Figs. 1 and 4) a definite siderophilic axostyle is visible, whereas in others (Fig. 2) it is very reduced and only found with much difficulty, and in yet others (Fig. 3), which show no indication of approaching division, it cannot be found after the most careful study. The axostyle ends posteriorly in the sharp projection of the posterior end of the body, but in forms which lack an axostyle this projection is not only present, but is sometimes accentuated (Figs. 2 and 3).

In the nucleus of forms about to divide (Figs. 5 and 6) the amount of siderophilic material increases and the place of the single karyosome is taken by a number of granules which ultimately form five discrete "chromosomes". In such stages the axostyle is either totally absent (Fig. 6) or very small (Fig. 5). The "chromosomes" become aggregated into a compact mass which is so dense that they can no longer be counted. The nuclear membrane is so thin that it cannot always be detected during division, but when visible

it becomes spindle-shaped though spindle fibres are never found. At the stage when the five "chromosomes" are present the blepharoplast divides and the daughters move apart but remain connected by a deeply staining centrodesmose. One daughter blepharoplast bears two of the free flagella, the other the remaining flagellum and the undulating membrane with its bordering flagellum. In some preparations (Fig. 7) the daughter blepharoplasts have divided again, the products remaining in apposition. The opposite granules of two such pairs are connected by the centrodesmose and are true centrosomes, whilst from the remaining two granules, which are the true basal granules, spring the flagella. How the new undulating membrane and its flagellum arises is not known, but in some individuals (Fig. 7) two membranes and flagella can be detected before the chromatin mass in the spindle-shaped nucleus divides. At the time when the division of the chromatin occurs the body of the flagellate is always rounded and axostyles are never seen. As the chromatin divides the daughter blepharoplasts move farther apart, the daughter nuclei following them (Figs. 9, 10 and 11), and the centrodesmose rapidly elongates. The body of the flagellate elongates in the same plane as the centrodesmose. The daughter nuclei at first contain a number of discrete granules, but later these come together to form a compact mass. New flagella, to make up the normal number, grow out from the daughter blepharoplasts. One curious phenomenon is that during the later stages of division the flagellum on the edge of the undulating membrane, and on the daughter membrane when visible, often projects freely for a short distance beyond the edge of the body. It has been impossible to determine the origin of the new axostyles. Nothing comparable with their development from the daughter blepharoplasts during the telophase, as seen in dividing trichomonads (Bishop, 1931 and 1932), has been found in this organism. The centrodesmose is sometimes absorbed before the two daughter flagellates separate. In the living flagellate the constriction round the middle and the actual division of the body is very rapid.

Multiple fission, or "double" individuals, such as occur in cultures of many species of *Trichomonas* (Bishop, 1931), are never found.

Cultures in all the types of media used were examined at all stages, and the debris at the bottom of old culture tubes from which the flagellates had disappeared was examined with the greatest care, but cysts were never found.

## VI. DISCUSSION

Although the living flagellate from the pond bears a striking resemblance to *Trichomonas*, both in general appearance and in movement, it differs from members of that genus in several structural characters. It differs from most trichomonads in the shortness of the undulating membrane and in the absence of a free projection of the bordering flagellum. When iodine preparations of this flagellate are compared with those of *T. batrachorum* or a macaque-human

strain[1] this difference is most striking, for the free end of the bordering flagellum shows up excellently in the two latter species whilst in the trichomonad-like flagellate from the pond it is not seen at all. The presence of this free end of the flagellum bordering the undulating membrane is not, however, invariable in *Trichomonas*, for when "*T. vaginalis*" is studied direct from the human vagina the undulating membrane is short (Reuling, 1921) and there is no free projection of the bordering flagellum. Mr Dobell tells me that this is also true for *Trichomonas* from the intestine when living experimentally in the vagina of *Macacus sinicus* (Dobell, 1934). But in "*T. vaginalis*", in contrast with the pond flagellate, a basal fibre is present. Andrews (1929) found that in the cultural forms the undulating membrane "grows longer and protrudes beyond the body as a free flagellum", and Dobell (1934) has proved that an intestinal trichomonad "can live in the vagina of *M. sinicus* as a typical '*T. vaginalis*' for several years at least". In this species therefore the form seems to be directly influenced by the environment. But in the pond flagellate change in the environment (*e.g.* from pond water to the various types of culture media) produced neither similar lengthening of the membrane nor a free termination of the bordering flagellum.

The flagellate from the pond differs from all true trichomonads in the absence of a basal fibre, and in the great reduction or total absence of an axostyle in many individuals. The siderophilic character of the axostyle, when present, appears—from Hinshaw's description (1926)—to be a character shared with *T. buccalis*. A hyaline axostyle, typical of *Trichomonas*, has never been seen in this flagellate from the pond.

Alexeieff (1910) described a flagellate from the oesophagus of the marine fish *Box boops* and tentatively named it *Trichomonas*? *legeri*. He later (1911) created a new genus, *Protrichomonas*, for it. This flagellate had three anterior flagella arising from a blepharoplast, and a nucleus with a single karyosome. An axostyle was not always present but when it existed it was siderophilic. *P. legeri* differs from the trichomonad-like flagellate from the pond in that not only is a basal fibre absent, but the undulating membrane and its associated flagellum are absent too.

*Tritrichomonas fecalis* (Cleveland, 1928), which resembles the flagellate from the pond in the variety of the cultural conditions under which it will live, has a hyaline axostyle, a basal fibre, and an undulating membrane reaching to the posterior end of the body with a long flagellum projecting freely from its end—all characteristics of a typical *Trichomonas*.

In the method of division the flagellate from the pond is similar to *T. hominis*, *T. batrachorum*, and *T. sanguisugae* (Bishop, 1931 and 1932), except that in *T. batrachorum* and *T. sanguisugae* six "chromosomes" are present whereas in the flagellate from the pond, at the only stage at which they can be

[1] The history of this *Trichomonas* is given by Dobell (1934). Mr Dobell most kindly isolated a strain for me to compare with my flagellate from the pond. I am greatly indebted to him for the gift of it and for the trouble he took in obtaining the strain in culture.

counted (the prophase), there are five, and that in the latter flagellate a basal fibre is absent and an axostyle is not always present.

The occurrence of *Trichomonas* outside any host, and its ability to live in vegetable media, have been described by several workers. Escomel (1913) claimed to have found *Trichomonas* in the drinking water of Arequipa, and to have cultivated *T. intestinalis* (=*T. hominis*), from dysenteric patients infected with the flagellate, in lettuce-stalk broth. He believed the flagellate he found in the drinking water to be similar to the trichomonads he found in the dysenteric stools. His account of the life history is dissimilar to that of any known trichomonad. It seems probable, as suggested by Dobell and O'Connor (1921), that the organism which he cultivated was a contaminant. Kuczynski (1914) saw trichomonads in muddy pools where lay the droppings of sheep and cows. He also states that he found trichomonads from the toad dividing in a 2-day-old culture made from their intestinal contents mixed with lettuce broth. Kuczynski also reports that Kunstler found trichomonads in brackish water, but Kunstler's account has not been accessible to me. *Tritrichomonas fecalis* was found by Cleveland (1928) in cultures from the faeces of one person, but he never found it in the newly passed faeces. *T. fecalis* grew in a suspension of the faeces in tap water, and also in hay infusions, sometimes in the company of free-living Protozoa. He found that *Trichomonas augusta* from frogs would also grow in suspensions of faeces in tap water. But though *T. fecalis* structurally was a typical trichomonad it differed from *T. augusta* in being less than half its size and in the absence of granules in its axostyle. Also though *T. fecalis* grew and multiplied indefinitely from −3 to +37° C. *T. augusta* died in a few hours at 37° C. Not only does the trichomonad-like flagellate from the pond differ from *T. fecalis* in structure (p. 252) but also in that it invariably dies at 37° C.

It has been shown that *Trichomonas batrachorum*, reared in culture, will live (Bishop, 1934), in some cases for months, in rain water, when the column of water is deep and there is absence of oxygen at the bottom. But dividing forms were not found, and the number of flagellates gradually decreased. Experiments were made to see if a pure strain of *T. batrachorum*, isolated from a toad on 8. x. 34 and grown in the part-solid part-liquid serum medium without starch (p. 248), would live in hay infusion, and if so whether any change would appear in its structure. A strain was transplanted into hay infusion on 23. x. 34. It multiplied in this medium, though slowly, and dividing forms were found. The richness of the subcultures varied, some being poor whilst others were fairly rich, but they never approached anywhere near the density of growth found in the hay infusion cultures of the flagellate from the pond. This hay infusion strain of *T. batrachorum* finally died out in its 10th subculture on 21. i. 35, the strain having lived in hay infusion for approximately 3 months. Two strains of *T. batrachorum* have been started by inoculating the intestinal content of toads into hay infusion. One strain, isolated on 7. xii. 34, is now in its 9th subculture, is fairly rich, and many dividing forms have been seen in it. *Blastocystis* soon died in the hay infusion, and, with the exception of *Entamoeba*

*ranarum*, none of the other parasitic Protozoa remained alive after the first subculture. Preparations, using the same technique (p. 249) as for the flagellate from the pond, were made of *T. batrachorum* grown for six, seven and eight generations in hay infusion, but no loss of the basal fibre nor shortening of the undulating membrane and its flagellum were found, and the axostyles had the normal, hyaline appearance. All attempts to grow *T. batrachorum* at 31° C. or at 37° C. have failed as previously (Bishop, 1934). Attempts to grow the macaque-human strain of *Trichomonas* (see footnote, p. 252) in hay infusion at 37° C. were made on four occasions. An inoculum from a rich culture of the *Trichomonas* grown on the "Ehs S." medium (Dobell, 1934) was put into a tube of hay infusion and incubated at 37° C. In all four experiments the flagellates were dead within 24 hours.

The fact that this trichomonad-like flagellate was never found directly in the pond water, but only after the water was centrifuged and allowed to stand, could be explained in two ways. Either the flagellates might have been present in the pond in an encysted state, and might therefore require time to hatch out, or they might have been very rare in the pond water and require a concentration, both of themselves and their food, before their appearance could be noticed and their rapid multiplication take place. The complete absence of cysts, from cultures of any age, and from pond water in which the flagellates had been numerous and from which they had died out, makes the former hypothesis very improbable.

The possibility that the flagellate might be the parasite, voided into the pond in faeces, of some host which frequented this water was considered. Its ability not only to live, but to multiply in pond water at room temperature, and its inability to live at 37° C. makes the hypothesis of its being a parasite of cattle, of a passing bird, or of some other warm-blooded animal, untenable. There is still a possibility that it may be the parasite of some cold-blooded inhabitant, or visitor, of the pond, but its ability to live so successfully under free-living conditions strongly points to its being a free-living flagellate. *Tritrichomonas fecalis*, though differing in points of structure, has many of the same cultural characters as my flagellate, but the origin of *T. fecalis* is wrapt in mystery, for Cleveland never actually saw it in freshly passed stools.

The flagellate from the pond differs from *Trichomonas* in the absence of a basal fibre and the frequent absence of an axostyle, and it differs from *Protrichomonas* in possessing a definite undulating membrane. I hope, in the near future, to study it more fully, as further information is needed about its habits and distribution before any definite decision on its systematic position can be made. So many new genera of Protozoa have been hastily created in the past, only to be proved synonyms, that I do not wish to add to the confusion. For the present, therefore, owing to its many *Trichomonas*-like characters, particularly the presence of an undulating membrane, I shall include it tentatively in that genus. There can be no question, so far as I am aware, that specifically it is new, and I shall therefore call it "*Trichomonas*" *keilini* n.sp.

## VII. SUMMARY

1. "*Trichomonas*" *keilini* n.sp. has three anterior flagella, and a short undulating membrane, whose bordering flagellum does not become free. The flagella arise from an anterior blepharoplast. There is a single nucleus, anteriorly placed, and a mouth, but no basal fibre to the undulating membrane. An axostyle may be present or absent, but when present it is siderophilic. The method of multiplication of "*T.*" *keilini* is similar to that of *T. hominis* and *T. batrachorum*.

2. "*T.*" *keilini* was found in water from a pond in Lincolnshire.

3. It has been cultivated in serum-saline media, in hay infusion, and in pond or rain water with boiled wheat grains, from 4 to 31° C.

4. Cysts have never been found.

I should like to record my gratitude to Prof. Keilin, F.R.S., who gave me the sample of water in which I first saw the organism, and who gave me encouragement throughout this study; and to Mr Clifford Dobell, F.R.S., for the gift of the macaque-human strain of *Trichomonas* and for so kindly reading through and criticising this manuscript.

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## EXPLANATION OF PLATE VII

All figures were drawn with an Abbé camera lucida under a 1/12 in. A. fluorite oil-immersion lens (Leitz) with a ×20 periplanetic eyepiece. The magnification of the figures is 3300 times. The flagellates were fixed with Bouin's fluid and stained with Heidenhain's iron haematoxylin.

Fig. 1. Flagellate with siderophilic axostyle, and nucleus at the resting stage containing a single karyosome.

Fig. 2. Nucleus contains two karyosomes. Axostyle almost invisible. Undulating membrane very much reduced.

Fig. 3. Flagellate at the resting stage. No axostyle.

Fig. 4. Flagellate as Fig. 1 but axostyle not so distinct.

Fig. 5. Flagellate preparing for division. Nucleus contains five "chromosome" granules. Axostyle very much reduced.

Fig. 6. Same as Fig. 5. No axostyle.

Fig. 7. Nucleus dividing. Blepharoplasts have divided and redivided. Centrodesmose joins the centrosomes. The new membrane and its flagellum is present.

Fig. 8. As Fig. 7 but the new membrane is not visible.

Figs. 9, 10, and 11. Examples of the final stages of division.

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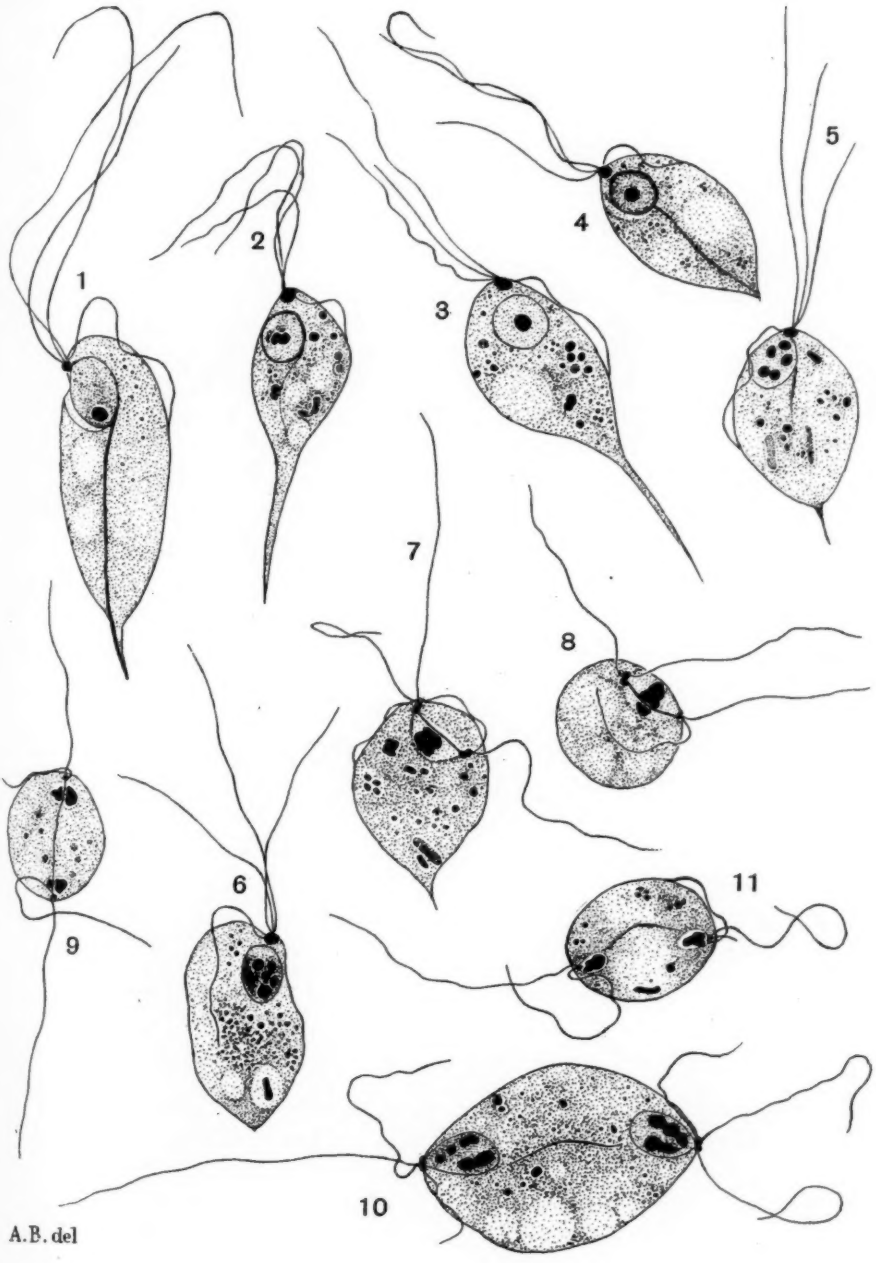
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# THE PERISPIRACULAR GLANDS OF MOSQUITO LARVAE

By D. KEILIN, P. TATE and M. VINCENT
*From the Molteno Institute, University of Cambridge*

(With 2 Figures in the Text)

Glandular cells in association with the spiracles of some Diptera have been described by a number of authors. They were first noted by Batelli (1879) in the larvae of *Eristalis tenax*. Bruno Wahl (1899) and, later, Dolley and Farris (1929), examined the glandular cells of *Eristalis tenax* and concluded that they secrete an oily or waxy substance which serves to protect the spiracles and peritreme from wetting and thus prevents the entrance of water into the spiracles and permits the larvae to hang from the surface film. Lowne (1893–5) described, in the imago of *Calliphora erythrocephala*, similar glands to which he attributed the function of secreting an oily or waxy substance which prevents wetting of the spiracles. Pantel (1901) found similar glands in entomophagous dipterous larvae. Keilin (1913) found these perispiracular glands in the larvae of Mycetophilids, Psychodids, Ptychopterids, Rhyphids and *Tricocera*, and stated that they are probably present in all dipterous larvae with functional spiracles and that their structure, the number of cells comprising them and the form of the intracellular ducts varies in different species. Krüger (1926) described glandular cells in connection with both the anterior and posterior spiracles of Syrphid larvae.

In connection with control measures against mosquito larvae much work has been done on the differential wetting of the spiracular region and the entrance of oils, soaps and other liquids of low surface tension into the tracheae (Freeborn and Atsatt, 1918; Hacker, 1925; Wigglesworth, 1930). Although the hydrofuge nature of the spiracular region was admitted the cause of it was unknown and glands secreting an oily substance such as those found in other dipterous larvae were not recorded.

We find that such perispiracular glands are present in mosquito larvae and are readily observed in living Culicine larvae (*Culex pipiens*, *Theobaldia annulata*, *Orthopodomyia pulchripalpis*). These glands are also present, but less easily observed, in Anopheline larvae (*Anopheles claviger* (*bifurcatus*) Mg.).

In living *Culex pipiens* L. the perispiracular glands show very clearly in newly moulted fourth instar larvae. In lateral view the glands appear as multicellular bodies enveloping the ventro-lateral region, and, to a less degree, the dorsal distal part of the felt chambers (Fig. 1 A, *v.g.*, *d.g.*). The cells forming the gland contain numerous highly refractile globules of varying size,

and hyaline streaks which are apparently intracellular clefts. In ventral view of the living larvae it is seen that large numbers of globules accumulate in rows in the angular areas formed by the apposition of the felt chambers and the median chitinised stirrup-shaped piece of Howard, Dyar and Knab (1912–17).

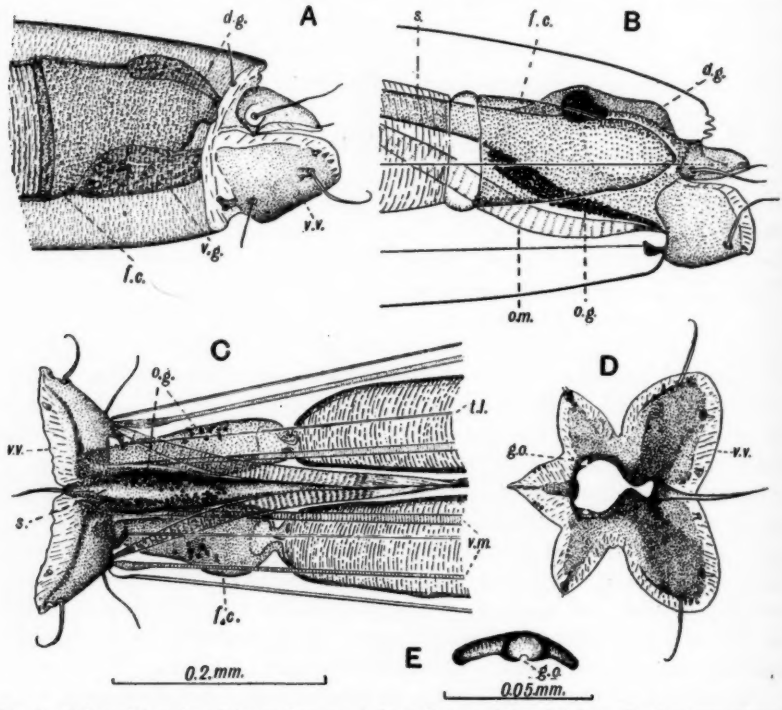


Fig. 1. *Culex pipiens* fourth instar larvae. A, lateral view of tip of siphon. B, the same view of the siphon of a larva fixed in Flemming's solution. Mounted in gum arabic. C, ventral view of distal part of siphon of a larva fixed in Flemming's solution and mounted in gum arabic. D, dorsal aspect of tip of siphon with the valves open. E, apex of a felt chamber. *d.g.* dorsal part of perispiracular gland; *f.c.* felt chamber; *g.o.* possible opening of perispiracular gland; *o.g.* oily globules of secretion stained with osmic acid; *o.m.* oblique muscles; *s.* stirrup-shaped piece; *t.l.* terminal ligaments of the ventral pair of chordotonal organs; *v.g.* ventral part of perispiracular gland; *v.m.* ventral muscles of siphon; *v.v.* ventral valves. (A, B, C, D same scale.)

In fixed and sectioned material the structure of the gland is best seen in transverse sections through the region of the siphon containing the felt chambers. The glandular cells occupy the area between the two felt chambers, closely embracing the ventral prolongation of the median stirrup-shaped piece, and extend ventrally and dorsally to envelop the ventro-lateral and dorso-lateral parts of the felt chambers (Fig. 2 A, B, *v.g.*, *d.g.*). The gland is largest about the middle of the felt chambers and gradually decreases in size towards

the base and the apex, terminating distally beneath the heavily chitinised basal areas of the ventral valves to which the glandular cells are closely applied. The cells comprising the gland have each a large nucleus and greatly vacuolated protoplasm in which there are also numerous clefts. The vacuoles are especially large and numerous in the median ventral and median dorsal regions in the angles between the stirrup-shaped piece and the felt chambers (Fig. 2 A, B, *v.*).

The glands stain red with Sudan III (in 70 per cent. alcohol), which also stains the internal lining of the felt chambers, thus indicating the presence of an oily secretion. In whole siphons fixed in Flemming's solution the glandular area is distinguished by the presence of densely stained globules which are especially obvious in two rows, one on each side, between the ventral part of the felt chambers and the stirrup, and also on each side of the dorso-lateral part of the felt chambers (Fig. 1 B, C, *o.g.*). In moulted cuticles oily globules which stain red with Sudan III are present in the distal part of the siphon along the dorsal and ventral parts of the stirrup.

The glands may also be seen in first, second and third instar larvae of *C. pipiens*, but in the first instar larvae the cells are less vacuolated and but little differentiated from the ordinary hypoderm cells. In other instars the activity of the cells may vary in different specimens, in some the cells are distended with numerous large globules while in others the globules may be small and less numerous.

In *Anopheles* (*A. claviger* Mg.) larvae the densely chitinised nature of the spiracular region and the presence of fat-body make observations on living material very difficult. In sections, however, glands similar to those of *Culex* larvae are seen ventral to the median chitinised plate of Imms (1908) (Fig. 2 C, *m.c.p.*). As in *Culex*, the gland is formed of modified hypoderm cells which have much vacuolated protoplasm containing numerous oily globules. In *Anopheles*, in longitudinal sections perpendicular to the median plate, the gland can be seen to extend from around and between the spiracular openings to near the posterior margin of the median plate (Fig. 2 C, *p.g.c.*). The cells forming the gland separate into two bands of tissue, one lying on each side of the muscles which are attached to the most heavily chitinised central part of the median plate, and again unite into a single band of tissue posterior to the muscles. Thus the gland is formed of bands of modified hypoderm cells, which, commencing at the spiracles, join together in front of and behind the muscles inserted into the median plate and, towards the lateral margins of the plate, merge with the unmodified hypoderm cells. Except for the different location of the gland in *Anopheles*, in correlation with the structural difference of the spiracular region, the gland resembles that of *Culex*. The apparent difference in position is readily understood when it is remembered that in *Culex* the gland is developed in close connection with the ventral prolongation of the stirrup-shaped piece which is homologous with the median plate of *Anopheles* (Christophers, 1922).

It is difficult to interpret staining reactions with Sudan III or Flemming's

solution in the case of *Anopheles* larvae owing to the large amount of fat-body present which stains densely and obscures the gland. In sections stained with Mann's methyl-blue-eosin, magenta-red-picro-indigo-carmine or haemalum-eosin, however, the glandular cells are clearly differentiated from the fat-body cells which contain very numerous small granules and which, moreover, are situated beneath the hypoderm whereas the glandular cells are directly

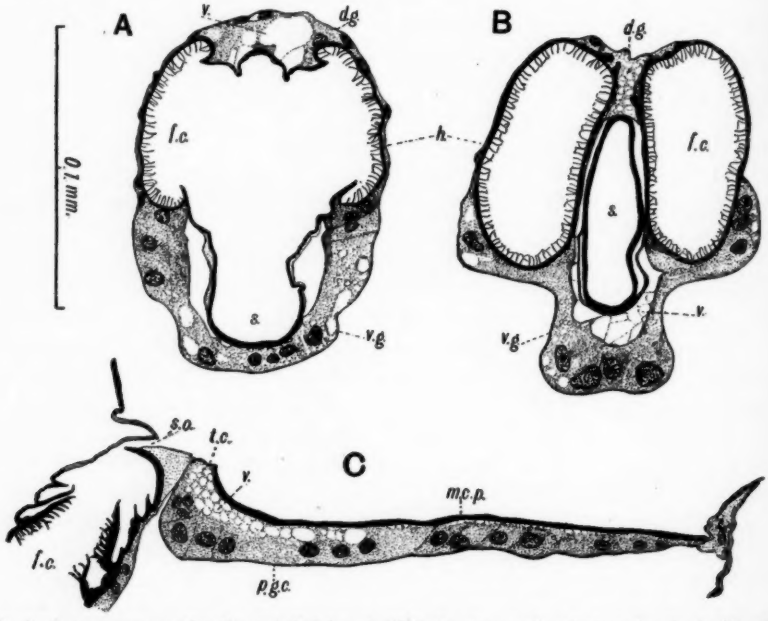


Fig. 2. A, transverse section through distal part of felt chambers and perispiracular gland of fourth instar larva of *Culex pipiens*. B, transverse section through the same larva about the middle of the felt chambers and of the perispiracular gland. C, longitudinal perpendicular section through spiracle, median chitinised plate and perispiracular gland of fourth instar larva of *Anopheles claviger*. (Fixative, Duboscq-Brazil. Stain, Mann's methyl-blue-eosin.) *d.g.* dorsal part of perispiracular gland; *f.c.* felt chamber; *h.* unmodified hypoderm; *m.c.p.* median chitinised plate; *p.g.c.* perispiracular gland cells; *t.c.* thin chitin; *s.* stirrup-shaped piece; *s.o.* spiracular opening; *v.* vacuoles in glandular cells; *v.g.* ventral part of perispiracular glands.

applied to the chitin. The glandular cells of *Anopheles* larvae in sections stained with Mann's stain or magenta-red-picro-indigo-carmine show the same staining reactions and structure as do those of *Culex* larvae.

The felt chambers in the larvae of *C. pipiens* terminate in small domed projections formed by the union of the ends of the inturned margin bordering the spiracular opening (Fig. 1 D, E, *g.o.*). These projections are devoid of felt and are covered on the internal faces with a fine membrane perforated medially by a narrow slit (Fig. 1 E, *g.o.*). The relation of these projections to the spiracles and felt chambers resembles that of the openings of the peri-

spiracular glands in some other dipterous larvae. Although we are unable to trace any actual connection between the perispiracular glands and these projections in *C. pipiens* larvae, it is possible that they mark the apertures through which the oily secretion is exuded. In *Anopheles* larvae such structures are not present and the secretion may pass out in the region of the thin chitin which extends between the posterior borders of the spiracles and the anterior margin of the median plate, or it may pass into the felt chambers through fine ducts.

The importance of the hydrofuge properties of the spiracles of mosquito larvae, especially as regards the utilisation of "oiling" as a control measure, has been so fully discussed by the authors previously cited and many others that it is not necessary to enlarge on it here. It is sufficient to point out that the presence of glands secreting an oily substance which we have described affords an explanation of the origin of this hydrofuge property of the spiracular region of mosquito larvae. The oily secretion can be wetted or dissolved by oils which are thus enabled to enter the spiracles and invade the tracheae. Reagents such as ether, chloroform and turpentine, by dissolving away the protective oily film, allow water to wet the spiracles and enter the tracheae.

## SUMMARY

1. Perispiracular glands are present in the larvae of *Anopheles* and *Culex*.

2. These glands secrete an oily substance which is responsible for the hydrofuge properties of the spiracular region of mosquito larvae.

3. The differential wetting of the spiracular region and the action of oil on mosquito larvae depend on the presence of this oily secretion.

ACKNOWLEDGMENT. We wish to express our thanks to Mr J. F. Marshall of the British Mosquito Control Station, Hayling Island, and to Mr T. T. Macan, Cambridge, for their kindness in supplying us with larvae of *Anopheles claviger*.

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# CHANGES IN THE COMPOSITION OF ADULT *CULEX PIPIENS* DURING HIBERNATION

By P. A. BUXTON

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(With 1 Figure in the Text)

THERE are conspicuous differences in respect of hibernation among the mosquitoes; for certain species, such as *Culex pipiens* and *Anopheles maculipennis*, hibernate as adults, but others, for instance *A. claviger* (*bifurcatus*), pass the winter as larvae. These differences have been the subject of many investigations and of much theoretical discussion; perusal of the literature suggests that we still lack a sufficient knowledge of the facts. It is known that an accumulation of fat in autumn is characteristic of those mosquitoes which hibernate as adults, as it is of several insects of other orders. But it seems that little is known of the quantity of fat stored, or of the rate at which it disappears as winter passes. It is my present purpose to set out certain observations on the composition of adult female *Culex pipiens* at different times during the period of hibernation. Later it may be possible to make an experimental study of the causes of hibernation and to discuss the relevant literature. But at the moment let it suffice to refer to the following papers, from which the reader may inform himself of the present state of knowledge and theory: de Boissezon (1930), Roubaud (1932), and Shlenova (1933). The paper by Hecht (1933) contains a valuable summary of the subject.

All the material here used was obtained in a cellar beneath a house about four miles from Tonbridge, Kent. The house is remote from any village, and if there is any difference between the urban and rural races of our mosquito, we can feel certain that our specimens were rural. In this cellar adult females were collected at intervals from September to April in the years 1930–4. They were posted to London, the dead mosquitoes removed, and the rest killed, counted and weighed immediately after their arrival. They were then dried at 105° C. to constant weight, after which they were treated with ether in a Soxhlet apparatus, everything which was dissolved by the ether being regarded as fat. The facts obtained are set out in Fig. 1, which gives mean monthly values (mg.) per female. For each month I examined from 2 to 5 collections with 40–100 females in each. The figures for three months at the beginning, middle and end of hibernation are also given in Table I. It is assumed that what is lost at 105° C. is water, and what dissolves in ether is fat: neither assumption is strictly accurate.

The following conclusions may be drawn from the figure and table:

(1) As hibernation proceeds, there is a gradual reduction in the female's total weight from over 3 to under 2 mg.

(2) As to fat, it might appear that there is an increase from September to October. The figures are based on eight samples collected during two separate years, and examination of the original data does not show a con-

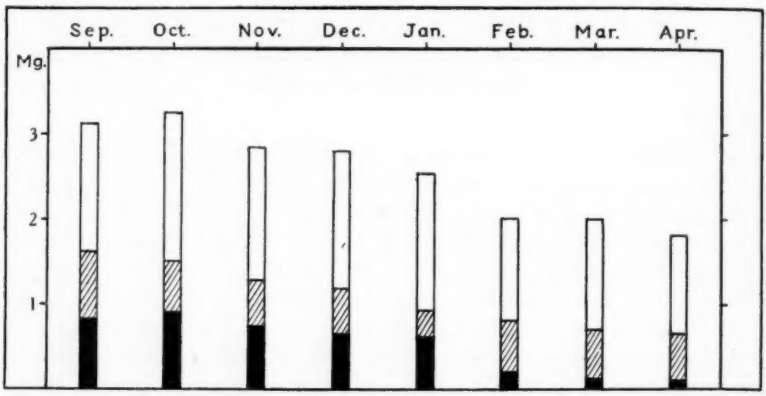


Fig. 1. Showing weight of water (unshaded), fat (black) and other solids (shaded) in an average female *Culex pipiens* at different times during hibernation.

Table I. *Showing amounts and proportions of certain substances in female* Culex pipiens *killed at different times during the course of hibernation* (*weights in mg.*).

| | mg. | | | |
|---|---|---|---|---|
| Month | Total | Water | Solids-not-fat | Fat |
| October | 3·26 | 1·76 | 0·59 | 0·91 |
| December | 2·80 | 1·63 | 0·51 | 0·66 |
| March | 2·05 | 1·33 | 0·59 | 0·13 |
| | | Proportions | | |
| October | 100 | 54·0 | 18·1 | 27·9 |
| December | 100 | 58·2 | 18·2 | 23·6 |
| March | 100 | 64·9 | 28·8 | 6·3 |
| October | — | 100 | 33·5 | 51·7 |
| December | — | 100 | 31·3 | 40·5 |
| March | — | 100 | 44·4 | 9·8 |

sistent rise or fall of fat during these two months, for there is considerable irregularity even within a single year. I conclude, therefore, that no significant difference between September and October has been demonstrated. Towards the end of hibernation, particularly in March and April, the figure for fat is very low, falling to about one-seventh of what it was in September and October. It is reasonable to assume that this residual ether-soluble fraction is not fat but other lipoids.

(3) The solids other than fat show remarkably little change in weight during the period of hibernation; indeed, the mean for October and for March is the same (0·59 mg. per female), and the figures for the different months all range between 0·51 and 0·59 mg., except for January, for which the figure is 0·40 mg.: the apparent anomaly may be disregarded, for only two January collections were examined, both in the same year.

(4) We may now consider the proportions of various substances present in the insect. As hibernation proceeds the weight of fat decreases more rapidly than that of water, the weight of solids remaining constant: it follows that in the insect as a whole the proportion of water rises during hibernation. But our interest is in the water balance of the living tissues, and we may therefore exclude fat because it has no effect upon osmotic forces. This has been done in Table I (last 3 lines), from which it appears that the proportion of solids other than fat rises greatly during hibernation; but it does not follow that the concentration of the tissue fluids rises, for it is quite possible that at the beginning of winter water is stored in the rectum, the oesophageal diverticula or some other space. It may be remembered that it was shown by Marshall and Staley (1932) that a proportion (8 out of 62) of females dissected in October and November have fluid as well as air in their diverticula: but in February and March none contain fluid. The same authors observed that, as hibernation progressed, there was an increase in the amount of air in the diverticula. We may assume that, as the fat disappears, the space which it occupied is partly filled by increasing the amount of air in the diverticula, so that the insect's loss of weight is greater than the reduction in its size.

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# *ANCHISTROTOS LAQUEUS* N.SP. A PARASITIC COPEPOD OF *SERRANUS CABRILLA*

By W. HAROLD LEIGH-SHARPE, M.Sc. (Lond.)

(With 3 Figures in the Text)

**Habitat and Material.** Seven specimens taken on the gills of *Serranus cabrilla* at Plymouth on 11. v. 1934 and kindly presented to me by Dr R. Gurney.

The genus *Anchistrotos* was erected by Brian 1906 (*A. gobii* on *Gobius capito*) for an Ergasilid Copepod resembling *Bomolochus* but which, in the female, presented two striking differences from the latter genus:

(1) The *maxillipedes* instead of being placed laterally to the maxillae as in the females of all the species of *Bomolochus* are located posteriorly to them as in males (Fig. 2).

(2) A *maxillary hook* is present (Figs. 1 and 2, *H*).

To these may be added:

(3) *Cephalothorax* bluntly rounded anteriorly, with a broadly rounded median projection.

(4) *Abdomen* of four segments.

A peculiarity of this new species, in addition to the details of the appendages, is that the *ovisacs* are short, scarcely longer than the abdomen, broad, with few large ova containing much yolk (Fig. 1).

**Body** (Fig. 1). Elongated. *Cephalothorax* bluntly rounded in front, nearly square in outline, with almost parallel sides, with a broadly rounded median projection anteriorly. *Third thoracic segment* nearly as wide as the cephalothorax, the others diminishing regularly in size. *Genital segment* much enlarged in the female. *Abdomen* of four segments decreasing in size posteriorly; abdominal appendages elongated, each bearing two long and two short setae.

**Appendages** (Figs. 1 and 2). *Antennules* six-articled, reflexed in all specimens so as to cover the maxillary hook, the three proximal articles widened and flattened, the four proximal articles densely setose, the terminal article capped by two long, and often one short, setae.

*Antennae* of the usual type, three-articled, terminating in three claws and a serrate plate, the basal article as long as the other two together which are flexed upon it, with roughened surfaces on the outer margin.

*Mandibles* undivided, abruptly curved, and ending in a strongly curved spine and two subsidiary ones.

*First maxillae* small flat plates bearing the three usual setae. The stout hook situated medially to the first maxilla is probably part of it but does not appear to be continuous with it.

*Second maxillae* two-articled, ending in two long spines, the posterior (ventral) of which is toothed.

*Maxillipedes* immediately posterior to the second maxillae, instead of being lateral to them, no distinct articulations but faintly indicative of four, of

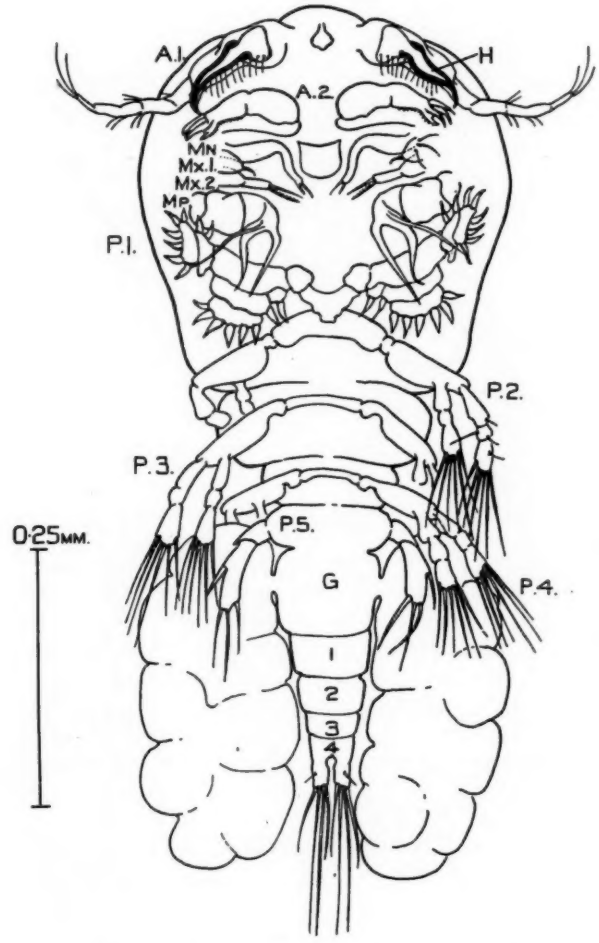


Fig. 1. *Anchistrotos laqueus* ♀, n.sp., ventral aspect.

which the second overlaps the third where the appendage is bent on itself, and the base may be fixed to the body wall. From the enlarged base arise two setae and a slightly sigmoid terminal spine serrated along one edge.

Four pairs of *Pereiopods* biramous, each ramus three-articled except the

first pair which is flattened, bears plumose setae and has each ramus two-articled.

Fifth pair of *Pereiopods* uniramous, two-articled, the long distal article flattened and bearing three apical and one lateral setae.

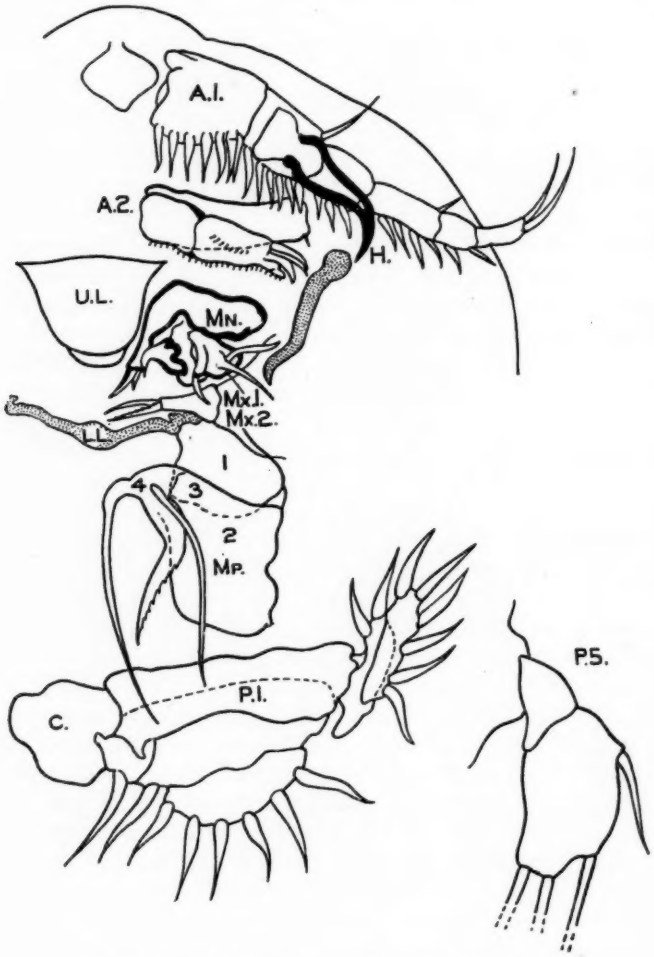


Fig. 2. *Anchistrotos laqueus* ♀, n.sp., anterior appendages.

The *maxillary hook* is long, stout, curved and prominent and appears to be concealed by the second and third articles of the antennule.

A figure is given (Fig. 3), for comparison, of the mouth parts of a typical *Bomolochus*, *B. bellones*, which is moderately common on *Belone acus* at

Plymouth. Here the maxillipedes are laterally situated to the maxillae. The setae on the first maxillae are plumose, and the appendages medial to it (*Pp.*) are usually regarded as mandibular palps.

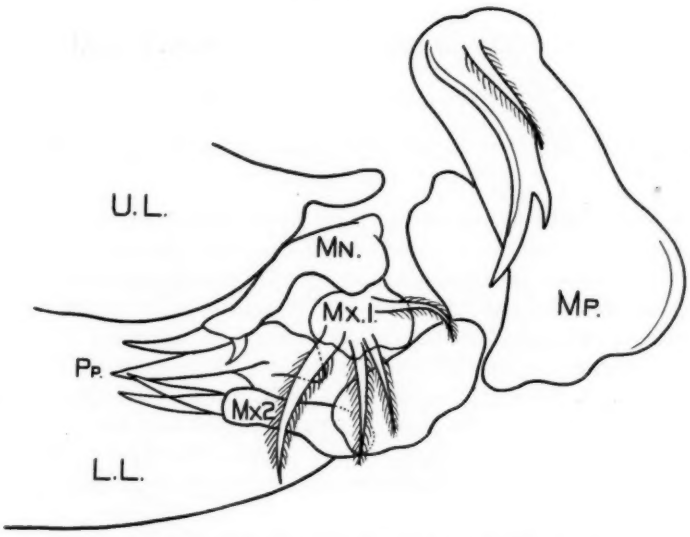


Fig. 3. *Bomolochus bellones* Burmeister, ♀, mouth parts.

The type and co-types (5 slides) are deposited in the British Museum.

My thanks are due to Dr C. L. Oakley for assistance in making out the appendages.

**Explanation of the Lettering of Figs. 1–3**

1–4 (Fig. 1) = abdomen; *A.* 1. = antennules; *A.* 2. = antennae; *C.* = coupler; *G.* = genital segment; *H.* = maxillary hook; *L.L.* = lower lip; *Mn.* = mandible; *Mp.* = maxillipede; *Mx.* 1. = first maxilla; *Mx.* 2. = second maxilla; *P.* 1.–5. = pereiopods; *Pp.* = mandibular palps; *U.L.* = upper lip.

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# TWO COPEPODS (*LERNAEENICUS*) PARASITIC ON *CLUPEA*

By W. HAROLD LEIGH-SHARPE, M.Sc. (Lond.)

(With 8 Figures in the Text)

## I. *Lernaeenicus spratta* (Sowerby) a parasitic Copepod of *Clupea spratta*

Since the publication of the original paper by Sowerby (1806) no investigator appears to have thoroughly examined *Lernaeenicus spratta*; even Scott and Scott (1913) in their monograph do not figure all the appendages.

**Habitat and Material.** A catch of 970 sprats taken at Hole's Hole, Plymouth, in the spring of 1934 yielded twenty-nine specimens of *L. spratta* (and one specimen of *L. encrasicholi* (Turton)), that is almost 3 per cent. were parasitised. Within the same period Dr R. Gurney examined a catch of an even larger number of sprats, over 1000, from the same place and obtained the same percentage of parasitism within a decimal. Scott and Scott's experience at Blackpool in 1910 was that 600 sprats yielded fourteen parasites, about 2·3 per cent.

The head of the parasite is completely embedded in the eye of the sprat and has to be carefully dissected out. A well-marked hole in the cornea permits the passage of the neck. The horns of the head form an anchor under the choroid coat which comes away with them. The antennae are buried in the retina and are frequently left behind on dissection; the animal feeds on the blood of the vascular choroid. The parasite causes partial or total blindness, and Scott and Scott record that there may be more than one parasite buried in one eye.

**Dimensions.** Length of head and neck 5·6 mm.; of trunk 8·3 mm.; of abdomen 0·75 mm.; of eggstrings 27·8 mm. Total length 41·7 mm.

**Body** (Fig. 1). Elongated, slender, divided into cephalothorax, neck, trunk and abdomen.

*Cephalothorax* enlarged, deep chocolate brown, globular, provided with two long stiff slender lateral horns projecting obliquely backwards.

*Neck* slender, elongated, enlarged through the bases of the swimming legs (pereiopods), then becoming filiform and chitinous, not twisted nor flexed, but with numerous prominent constrictions giving a moniliform appearance which distinguishes this from all other known species.

*Trunk* cylindrical and straight, moderately elongated and increasing slightly in diameter posteriorly. Pale brown.

*Abdomen* straight, narrower than trunk, extremely small but clearly defined, one-eleventh the length of the trunk.

*Eggstrings* filiform and very long, twice the length of the body. *Eggs* uniseriate and strongly flattened.

**Appendages** (Fig. 2). *Antennules* short, turned back along the surface of the head, and consisting of a distinct concave socket in which moves the re-

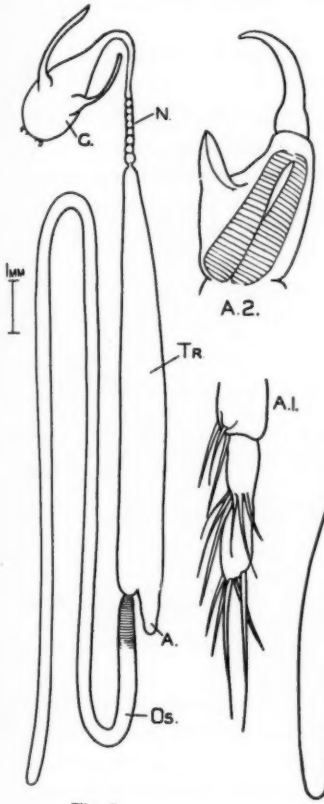


Fig. 1

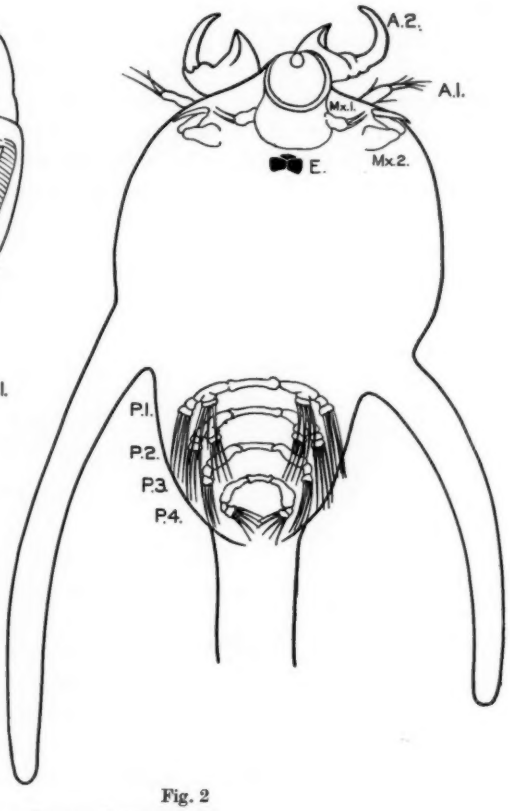


Fig. 2

*Lernaeenicus spratta* ♀

Fig. 1. Entire animal. *C.* = cephalothorax; *N.* = neck; *Tr.* = trunk; *A.* = abdomen; *Os.* = egg-strings.

Fig. 2. Enlarged view of anterior end in ventral aspect, and appendages. *A.* 1. = antennule; *A.* 2. = antenna; *E.* = eye; *Mx.* 1. = first maxilla; *Mx.* 2. = second maxilla; *P.* 1.–4. = pereio pods.

mainder of the appendage, which is indistinctly separable into three portions which are not separate articles, in this agreeing with most species. All the articles are extremely setigerous.

*Antennae* very large, standing well out in front of the head, strongly chelate, no attachment plates in front, two-articled and terminating in a very

long, slender flexible claw, with an enlarged base, and a stout projection having a terminal socket into which the claw shuts down.

The *Mouth cone* (Fig. 4) is anteriorly placed and strongly supported by struts.

The *Mandibles* (Fig. 4) are stout, styliform and irregular toothed, the projections varying in number.

The *first Maxillae* (Fig. 3) are one-articled, terminating in two long equal setae.

This appendage has been overlooked by all previous investigators and only recorded in *L. cerberus* (Leigh-Sharpe, 1927) where it is similar.

The *second Maxillae* (Fig. 3) are three-articled; the proximal article stout, and the terminal article a claw which shuts down against the middle article.

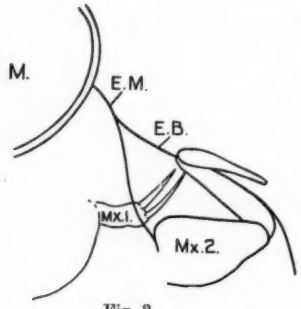


Fig. 3

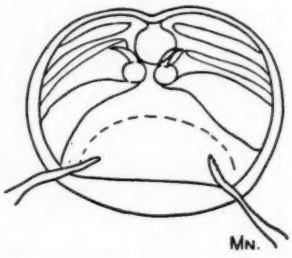


Fig. 4

*Lernaeenicus spratta* ♀

Fig. 3. *E.M.* = edge of mouth cone; *E.B.* = edge of body; *Mx.* 1. = first maxilla; *Mx.* 2. = second maxilla; *M* = mouth.

Fig. 4. Mouth cone. *Mn.* = mandible.

*Pereiopods* (Fig. 2) comprise four pairs situated upon the dilated portion of the ventral surface of the neck. These diminish in size regularly backward, the first two pairs being biramous, and the last two pairs uniramous. All the rami are two-articled. The basal articles are elongated, and the articles of the rami are of almost equal size, the distal one being tipped with four setae, except the last pair which has five.

The tripartite *eye* of the larva persists in the adult, and this is the first record of its appearance in a British species (Fig. 2, *E*). It is buried deeply in the tissues over the base of the oesophagus and has been covered by the surrounding tissues during the growth of the horns.

The oesophagus is short, straight, and inclined to the body axis; stomach without lateral lobes but with one or two convolutions passing gradually into the intestine, which is very narrow in the neck, but wider than the stomach in the trunk, abruptly contracted at the posterior end of the abdomen into a narrow rectum.

*Ovaries* paired, short, wide, strongly flattened between the intestine and

the dorsal body wall at the anterior end of the trunk. *Oviducts* passing round the intestine to the ventral surface and then straight back to the vulvae. Cement glands slender, cylindrical.

## II. *LERNAEENICUS ENCRASICHOLI* (TURTON 1807) A PARASITIC COPEPOD OF *CLUPEA* SPP.

**Habitat and Material.** As already stated a catch of 970 sprats taken at Hole's Hole, Plymouth, in the spring of 1934 yielded but one *Lernaeenicus encrasicholi*. A. Scott (1907) reports only three parasites from a large catch

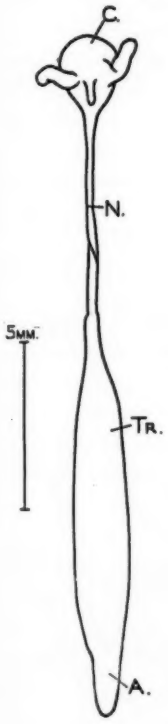


Fig. 5

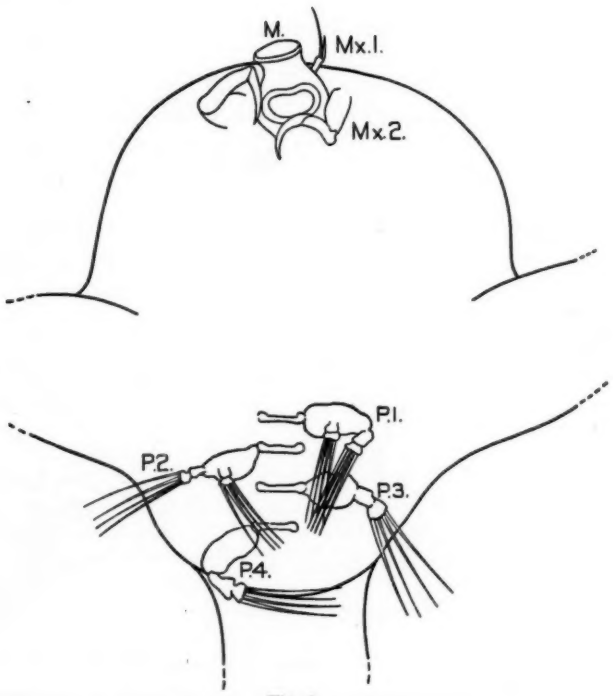


Fig. 6

*Lernaeenicus encrasicholi* ♀

Fig. 5. Entire animal. *C.* = cephalothorax; *N.* = neck; *Tr.* = trunk; *A.* = abdomen.

Fig. 6. Enlarged view of anterior end in ventral aspect, lettering as in Figs. 2, 3 and 8. *P.* 1.–4. = pereiopods.

of sprats at Blackpool, 19. ii. 1906. A broken specimen taken by Bassett-Smith from *Clupea alosa* at Plymouth was considered by him to belong to this species. Turton (1807) found it frequently on the bodies of the anchovy, *Engraulis encrasicholus*, and the sprat in Swansea Bay.

This species is a muscle borer and is usually found buried in the lateral myomeres of the host. The head of the parasite penetrates into the coelom and remains in close proximity to the principal blood vessels.

*L. encrasicholi* can be distinguished from *L. spratta* by the following characters:

(1) The horns instead of pointing obliquely backwards stand out nearly at right angles to the cephalothorax.

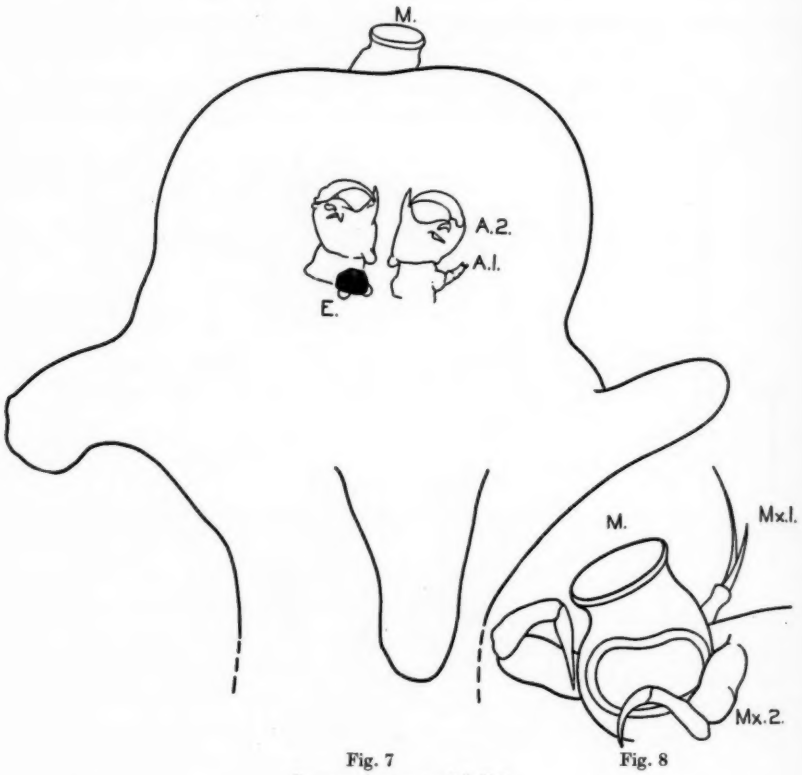


Fig. 7 Fig. 8

*Lernaeenicus encrasicholi* ♀

Fig. 7. Cephalothorax in dorsal aspect. *M.* = mouth cone; *A.* 1. = antennule; *A.* 2. = antenna; *E.* = eye.

Fig. 8. Mouth parts. *M.* = mouth cone; *Mx.* 1. = first maxilla; *Mx.* 2. = second maxilla.

(2) The neck is smooth, *i.e.* without the constrictions so characteristic of the latter species.

(3) It is found embedded in the body of the host and not in the eye.

**Dimensions.** Length of head and neck 6·6 mm.; of trunk 8 mm.; of abdomen 1·5 mm. Total length 16·1 mm. Eggstrings lacking.

**Body** (Fig. 5). Elongated, slender, divided into cephalothorax, neck, trunk and abdomen.

*Cephalothorax* (Figs. 5 and 7) enlarged, globular, provided with two dorso-lateral, short, stout, soft rounded horns with curled apices and projecting at right angles, also a third small, narrow, white dorsal horn overlooked by Scott and Scott (1913).

*Neck* (Fig. 5) slender, elongated, enlarged through the bases of the pereiopods, then becoming filiform, straight, without constrictions. A barely perceptible constriction separates it from the *trunk* which is shorter and broader in proportion than in *L. spratta*.

*Abdomen* straight, almost as broad as trunk, very small, between one-fifth and one-sixth the length of the trunk.

**Appendages** (Figs. 6, 7 and 8). *Antennules* small and only made out with difficulty.

*Antennae* resembling those of *L. spratta* but instead of projecting prominently anteriorly they are placed far back on the cephalothorax dorsally (Fig. 7).

The *first Maxillae* (Fig. 8) are one-articled with a distal enlargement terminating in two unequal setae.

The *second Maxillae* (Fig. 8) are three-articled, the proximal oval and stout and the terminal article a sickle-shaped claw.

*Pereiopods* (Fig. 6) in all details as in *L. spratta*, but the basal articles are much inflated and the setae very elongated; the fourth pair with only four setae.

The *eye* is present (Fig. 7) but appears unlike that of *L. spratta*, apparently single with two colourless spheres posteriorly.

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* In this paper the names of the first and second maxillae are probably reversed.

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# ON SOME LARVAL TREMATODE PARASITES OF THE MUSSEL (*MYTILUS EDULIS*) AND THE COCKLE (*CARDIUM EDULE*)

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(With 5 Figures in the Text)

In this paper an account is given of new forms discovered by the writer during a survey of the parasites of mussels and cockles, obtained chiefly from the estuary at Conway, but also from other beds on the North Wales coast. The trematode parasites of shellfish in this area have not previously been dealt with, although several forms have been described from the Liverpool Bay area by the late Prof. Johnstone and his colleagues. Few additions have been made to our knowledge of the early stages of British marine Trematodes since the publication of a series of papers by Lebour (1912) and Nicoll (1914).

## I. Orange sickness of mussels (*Cercaria tenuans* (sp. inq.))

The greater part of the material from which this parasite is described was obtained on October 9th and 10th, 1933, when an unusual mortality occurred among the mussels brought to the Conway installation for purification. Between 900 and 1000 mussels died and gaped, but not more than 300–400 were examined and six infected specimens were obtained. The infected specimens were found without difficulty, as the mantle is a vivid marigold to blood orange due to the presence of innumerable sporocysts. Although about 1000 mussels have been opened by the writer during the course of his investigations only one example of an orange-sick mussel has been obtained in this way. That this parasite was much more prevalent in the early years of the Conway installation seems certain, as Dr Dodgson tells me that at one time it was possible to obtain specimens by inspecting the few dead mussels which are picked out of the tanks during the normal process of purification.

The identity of the form now described with that previously found is confirmed by some sketches made at the time by Mr H. H. Goodchild.

### *Sporocyst*

The sporocysts (Fig. 1) infest all the tissues of the body with the exception of the foot, and in particular the mantle. Here the parasites lie in close contact with each other, while the mantle tissue itself, and in fact the whole mussel, is of transparent thinness. The size of the mussels infected varied from over 3 in. down to just 2 in., the regulation size below which mussels may not be put into

the tanks. The sporocysts are oval, moderately contractile, and vary considerably in size. The average dimensions of a sporocyst containing fully developed and active cercariae are about 1·1 × 0·45 mm. The sporocysts are bright orange

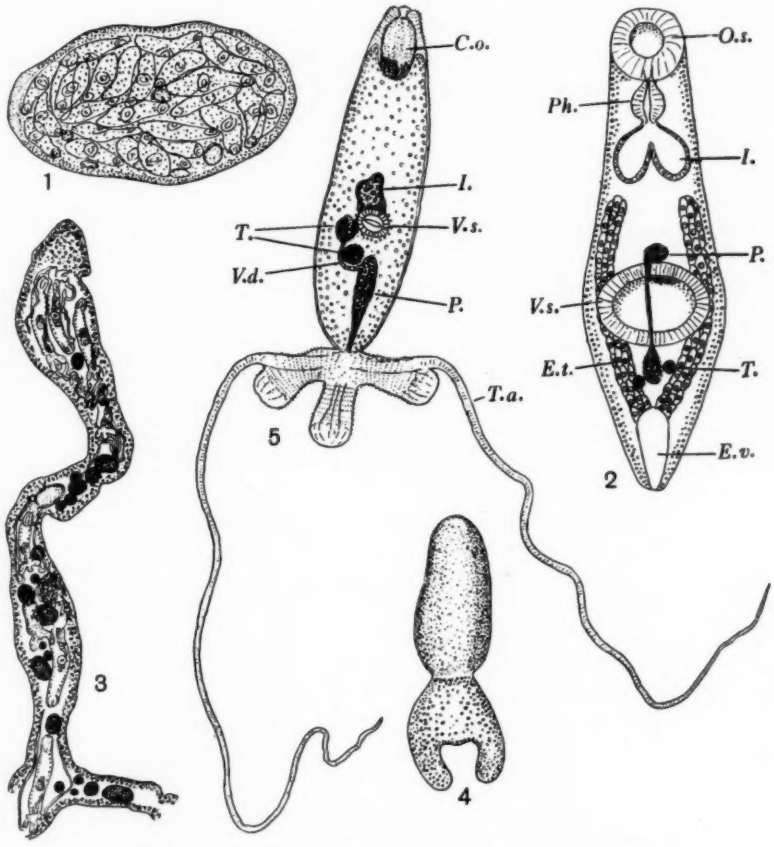


Fig. 1. *Cercaria tenuans*. Ripe sporocyst with cercariae. ×40.
Fig. 2. *Cercaria tenuans*. ×230.
Fig. 3. *Bucephalus mytili*. Sporocyst. ×40.
Fig. 4. *Bucephalus mytili*. Early cercaria. ×200.
Fig. 5. *Bucephalus mytili*. ×200.

**Lettering:** *E.t.* Excretory trunk, *E.v.* Excretory vesicle, *I.* Intestine, *O.s.* Oral sucker, *P.* Penis, *Ph.* Pharynx, *V.s.* Ventral sucker, *C.o.* Cystogenous organ, *T.* Testes, *T.a.* Tail appendage, *V.d.* Vas deferens.

and very thin walled, the heaving mass of cercariae being plainly visible through the walls. The orange pigment is arranged in spots of measurable size, the rest of the wall of the sporocyst being colourless. There seems to be a slight differentiation of the sporocyst at the anterior end, where a greyish, somewhat

thickened rim is visible, which is in a constant explorative motion, rather resembling a turbellarian. The whole sporocyst has also a constant wriggling motion. Most of the larger sporocysts contained numerous active cercariae, but early sporocysts were also seen. These contained either a densely granular fluid or round masses of cells, which are early cercariae. In one case where young sporocysts were found, the mantle of the mussel was studded with dark granular deposits, but this may have been accidental. The pigmentation of younger sporocysts is not so intense as in mature examples.

On March 15th, 1934, a mussel was obtained with a sample from Talyfoel, Anglesey, which contained a small number of orange sporocysts in the digestive gland. These were comparable with the sporocysts found in orange-sick mussels, but contained not cercariae but daughter sporocysts. If, as seems likely, this is a stage in orange sickness, then it shows that the primary infection probably occurs in the digestive gland.

### *Cercaria*

The length of the largest cercariae (Fig. 2) seen, when fully extended, was 0·3 mm. The cercariae were very active for a short time after pressing out from the sporocysts, but then became immobile very quickly, some surviving a few minutes. The anterior end of the cercaria is very extensible. The oral sucker is 0·05 mm. in diameter and the ventral sucker 0·07 mm. The suckers are very deep. The centre of the ventral sucker is 0·18 mm. from the anterior end. The skin is unspined but transversely wrinkled at the anterior end. The pharynx is well marked and there is a short prepharynx. There are two short, rounded intestinal diverticula to be seen only in stained specimens. The excretory trunks are large and end bluntly slightly less than half-way between the ventral and oral suckers; there is a large pear-shaped excretory vesicle into which the lateral trunks open. In stained cercariae the rudiments of the male reproductive organs can be made out with some difficulty. These consist of a pair of small globular testes placed one on each side of a prominent pyriform organ just behind the ventral sucker. A duct leads from the narrow end of the pyriform organ to what is presumably the penis, a spherical mass of cells situated just anterior to the ventral sucker.

This Trematode is mentioned by Miss Atkins (1931) as occurring in 2·16 per cent. of the mussels she examined from Padstow, Devon. From the very brief description that she gives there can be no doubt that it is the same form. It also occurred in one out of a batch of mussels brought from Boston, Lincs., to Conway for experimental purposes. Thus, although it has not been previously described, it is probably widespread in its distribution.

## II. A NEW *BUCEPHALUS* (*B. MYTILI*) FROM THE MUSSEL (*MYTILUS EDULIS*)

This parasite has occurred twice in the material examined. In the first instance in one out of a batch used for experimental purposes, obtained originally from the purification tanks at Conway, and secondly in a batch opened from the same source. Although nearly one thousand mussels have been opened by the writer during experimental and other work, these are the only examples of this parasite yet found. In the course of a study of the abnormalities present in mussel gills, Miss Atkins examined more than ten thousand mussels from Padstow, and she notes seven examples of infection by the sporocysts and cercariae of a species of *Gasterostomum*. Apparently these were not critically examined.

The cercariae occurred in long, thread-like, branched sporocysts (Fig. 3), inextricably tangled together and riddling the digestive gland gonad and mantle. In the mantle and on the surface of the body the pale yellow sporocysts were easily seen. The sporocysts contain cercariae in all stages of development.

The cercariae develop from dense rounded masses of cells which later become differentiated into an oval head portion and a triangular posterior part with short processes, which later forms the tail (Fig. 4). More advanced stages show the typical long tail processes which in fully developed cercariae are colourless.

The body of the fully developed *Bucephalus* (Fig. 5) is about 0·26–0·27 mm. long, while the tail processes may be nearly twice the length of the body. At the anterior end of the body is the so-called cystogenous organ, rather like a sucker in appearance and measuring about $0{\cdot}04 \times 0{\cdot}03$ mm. The ventral sucker, 0·025 mm. diameter, is situated behind the middle of the body 0·16–0·17 mm. from the anterior end. The intestine is single and sac-like, projecting forwards in most examples from the mouth, which is situated in the middle of the ventral sucker. There are a few rows of very minute spines around the anterior end.

The base of the tail is divided into a median and two lateral lobes. From the anterior surface of the latter the tail processes spring. The base of the tail is covered with minute spines giving it a striated appearance. The tail processes are very easily detached from the tail.

The male reproductive organs are very well developed and consist of a pair of spherical testes placed one behind the other, just posterior to the ventral sucker, and a vas deferens leading from them to a prominent penis, opening at the posterior tip of the body. No excretory vesicle could be seen.

As far as the writer is aware, the only British forms of *Bucephalus* are those described by Lebour (1912) and Johnstone (1921) under the names of *B. haimeanus* from the oyster and cockle and *B. syndosmyae* from *Syndosmya alba*. The form here described differs from the former in the shape of the tail and the position of the sucker, and from the latter in the form of the tail, the size of the

sucker, and the branching sporocysts, while the distribution of spines on the body and tail is also somewhat different. The writer was fortunate in having fresh material of *Bucephalus haimeanus* from an infected cockle at hand with which to make a comparison. There can be little doubt that the species described here is distinct. Several species of gasteromatous Trematodes have been described from British seas (see Sprehn, 1933) and the identity of this *Bucephalus* with a species of *Prosorhyncus* may yet be established.

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# NEW GENERA OF *MALLOPHAGA*. I

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(With 4 Figures in the Text)

A new genus is here described for the reception of three species of *Mallophaga*, belonging to the family Philopteridae, which have up to the present been listed under two separate genera, representing two subfamilies. The hosts of the three species under consideration all belong to the Charadriformes. For the present, three species only are included in this new genus, but it is probable that certain other already described species may ultimately have to be transferred to it.

**Rhynonirmus** n.gen.

*Head.* Variable in shape; strongly thickened along clypeal edge; signature indefinite though an irregular transverse clypeal suture is present; mouth parts unmodified; oral fossa remote, transverse, rounded, oblong. Trabeculae distinct, not exceeding the first antennal joint, generally much shorter. Heterocerous. Occipital edge nearly straight.

*Chaetotaxy*

Eight lateral clypeal bristles (4, 4), one minute at trabecula. Behind antenna three long bristles, viz. one at eye, one about one-half and one before postero-lateral angle, one minute bristle between the two last mentioned and two minute at angle, eight (4, 4) short, fine bristles on upper surface, viz. one at suture, one opposite trabecula, one behind antenna, one before occipital edge; ventrally, six (3, 3) short fine bristles along posterior edge of clypeal band, and one long ventral hair at insertion of antenna, also two (together) from underside of antennal band shortly before and one (minute) at the trabecula.

*Thorax.* Metathorax very wide angled posteriorly.

*Abdomen.* First tergite with sides divergent; tergites transversely continuous, transparent near spiracles. Tergites two to six meniscus shaped, and the intertergital membrane becomes strongly chitinised in fully adult specimens. Pleurites simple. Chaetotaxy, one dorsal and one ventral row.

♂. *Genitalia.* Basal plate widest posteriorly. Mesosome triangular, shortly produced posteriorly; parameres excessively thin towards inner edge, and closely fitting the mesosome. The latter bears one pair of minute bristles on the wider portion, and three pairs apically. A sac is present.

*Genotype. Rhynonirmus infuscatus* (*Lipeurus infuscatus* Osborn 1896).

**Rhynonirmus infuscatus** (Osborn) 1896

*Lipeurus infuscatus* Osborn, *Bull. U.S. Bur. Ent.* p. 234, Pl. 2, figs. *e, f.*
*Lipeurus infuscatus* Kellogg, *Gen. Insect.*, Mallophaga, 1908, p. 40.
*Esthiopterum infuscatum* Harrison, *Parasitology*, 1916, **9**, p. 136.

*Type hosts. Philohela minor* (Gmelin) and *Bartramia longicauda* (Bechstein).

*Specimens examined.* All off *Bartramia longicauda* (Bechstein) from the following localities: 2 ♀♀, Bay of Honduras, Ruatan Island, 1886 (G. F. Ganmer Coll.); 5 ♀♀ 2 ♂♂, Isthmus of Panama (McLeannan); 1 ♀, U.S.A., Florida, Tarpon Springs, 20. iii. 1889 (W. E. D. Scott Coll.); 1♀ 1♂, 49th parl., 4. vi. 1874, N. A. Boundary Comm. (M. Dawson); 1 ♂, Brazil, Trisanga, 7. xii. 1822.

*Note.* Although I have not seen Osborn's type material or any specimens taken off *Philohela minor* (Gmelin) I feel convinced that the specimens listed above are identical with Osborn's species.

*Description*

Length: ♀ 1·9–2·05 mm.; ♂ 1·69–1·73 mm. Breadth (5th seg.): ♀ 0·49–0·57 mm.; ♂ 0·40–0·44 mm.

♀. *Head.* Forehead circumfasciate; just longer than hindhead, clypeal band medianly about twice as broad as antennal band. Antennal emargination shallow. Antennae 20, 30, 20, 15, 23; the first joint nearly as broad as long, and about one-half wider than the others. Trabecula less than half as long as first joint.

*Thorax.* Prothorax transverse (2:1); one strong bristle at postero-lateral angle. Mesometathorax (8:5) with bristles 5, 5, the third and fourth being strongest. A minute bristle at side in front of first bristle. Sternal plate kite-shaped, widest at two-fifths from anterior angle with a pair of bristles anteriorly, another pair posteriorly.

*Abdomen.* About one-half longer than head plus thorax, tergite 1 divided medianly, tergite 2 divided to beyond one-half, tergite 3 to nearly one-half, tergites 4–5 or 6 merely notched.

♂ similar to ♀. *Head* (see Fig. 1*a*). Antenna (see Fig. 1*b*), apical edge of third joint oblique, but the actual appendage very short.

*Abdomen.* About one-third longer than rest of body. The secondary chitinisations between tergites 4 and 6 are broad and strongly developed.

*Chaetotaxy*

*Abdomen.* Upper surface and sides.

*In both sexes* tergite 1 bears 2 bristles (1, 1) just behind the posterior margin of the metathorax, and on all tergites 2–8 is a posterior row of bristles (2, 2); not including the post spiracular bristles. The latter on tergites 3–5 are longer; on tergites 6–7 short and fine; on tergite 8 rather short, fine, and displaced into association with the lateral bristles.

*In the* ♀ the posterior row on tergites 5–8 may contain one or two extra

bristles. Tergite 9 has two (1, 1) rather short and fine median bristles just behind the anterior edge, and a second pair (1, 1) long and stout near the lateral margin just behind one half. Lateral bristles tergites 4–5, one fine, tergites 6–7, two and three respectively. Tergite 8, four bristles (two long, two short). Tergite 9, two (long and short) at antero-lateral angle, a similar pair behind one half, and one very short before apex of terminal lobe.

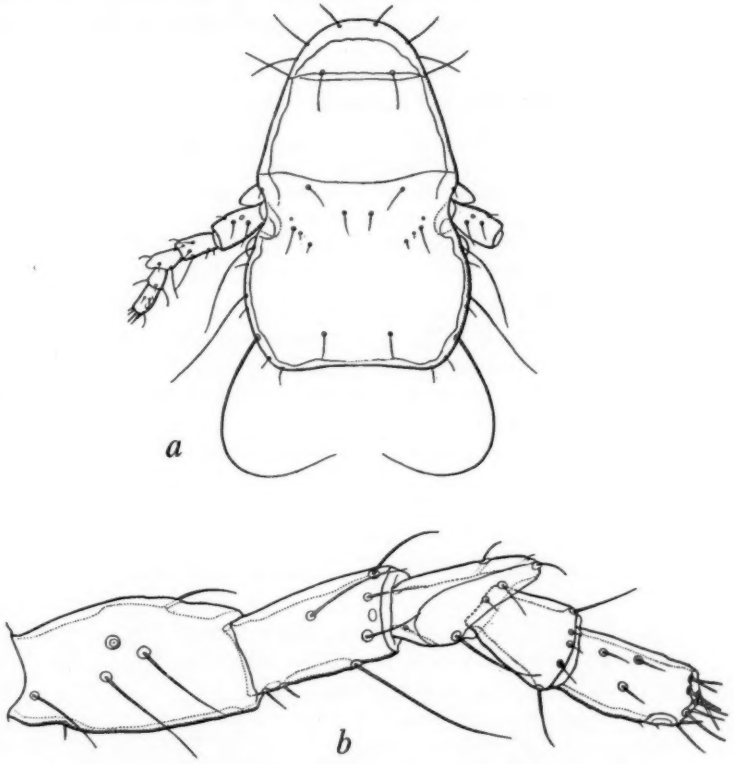


Fig. 1. *Rhynonirmus infuscatus* (Osborn). *a*, head of male; *b*, antenna of male.

*In the* ♂ the posterior row apparently never has any extra bristles. Tergites 8–9, one pair fine bristles. Anteriorly, another posteriorly, and wider apart with four (2, 2) on suture between tergites 9 and 10. The latter with a minute bristle on each apical lobe terminally.

Lateral bristles, tergite 8, two (long and short) at antero-lateral angle, and one very long and strong postero-laterally.

*Under surface. In both sexes* sternites 2–6 bear six bristles of which the middle pair on sternites 2 and 3 are placed anteriorly (so that these sclerites have two rows). *In the* ♀ sternites 7 and 8 with two (1, 1) bristles each, and two

more (belonging to sternite 7) on the suture between them. Sternite 9, three short stiff bristles behind the vulva.

*In the* ♂, sternite 7, four bristles; sternite 8, two bristles; sternite 9, two (1, 1) with two minute anteriorly at the base of each of the longer bristles, and ten minute bristles in two patches near posterior edge. Sternite 10, four bristles (2, 2) transversely placed, and nearly decussating.

♂. *Genitalia* (see Fig. 4*a*). Paramere straight edged internally, and shorter than mesosome; six to seven times as long as broad, and gradually contracted apically.

### **Rhynonirmus scolopacis** (Denny) 1842

*Nirmus scolopacis* Denny, *Anoplur. Brit.* p. 149, Pl. 11, f. 8.
*Nirmus tristis* Giebel, *Ins. Epiz.* 1874, p. 168.
*Nirmus truncatus* Nitzsch, in Giebel, *Z. ges. Nat.* 1866, **28**, p. 375.
*Nirmus truncatus* Giebel, *Ins. Epiz.* 1874, p. 168.
*Nirmus truncatus* Piaget, *Les Pédiculines*, 1880, p. 178, Pl. xv, f. 2.
*Degeeriella scolopacis* Harrison, *Parasitology*, 1916, **9**, p. 122.

*Type host. Capella gallinago gallinago* (Linn.).

*Specimens examined.* Denny's type material consisting of 1 ♀ and one immature off *Scolopax gallinago* (=*C. g. gallinago*), Britain; 1 ♂ and 1 ♀ off *Gallinago coelestris* Frenz. (=*C. g. gallinago*), Shetland Islands, N. Mavine, Bardister, 30. xi. 1912 (J. Waterston Coll.).

*Note.* I am accepting Harrison's synonymy as I have not seen any of the types of the other authors cited.

#### *Description*

Length: ♀ 1·76 mm.; ♂ 1·74 mm. Breadth (5th seg.): ♀ 0·43 mm.; ♂ 0·38 mm.

♀. *Head.* Forehead one-fourth longer than hindhead, clypeal band three and a half times as broad as antennal band. Antenna 20, 25, 15, 15, 20. Trabecula about half as long as first joint.

*Abdomen.* Nearly one-half longer than the rest of body.

♂. *Head* (see Fig. 2*a*). General proportions of fore-, hindhead and abdomen to rest of body as in ♀. Antenna (see Fig. 2*b*), third joint apically uniform.

#### *Chaetotaxy*

*Abdomen.* ♀. Almost identical with *infuscatus* (Osborn), but no extra bristles in posterior row on tergites 5–8. The sternites tend to have fewer bristles, *e.g.* sternites 1 and 2 have four each only.

♂. Sternites 1–6 with four bristles, sternite 9 with up to seven minute bristles.

♂. *Genitalia* (see Fig. 4*c*). Paramere straight edged internally, and shorter than mesosome; nearly five times as long as broad, and rather abruptly narrowed distally.

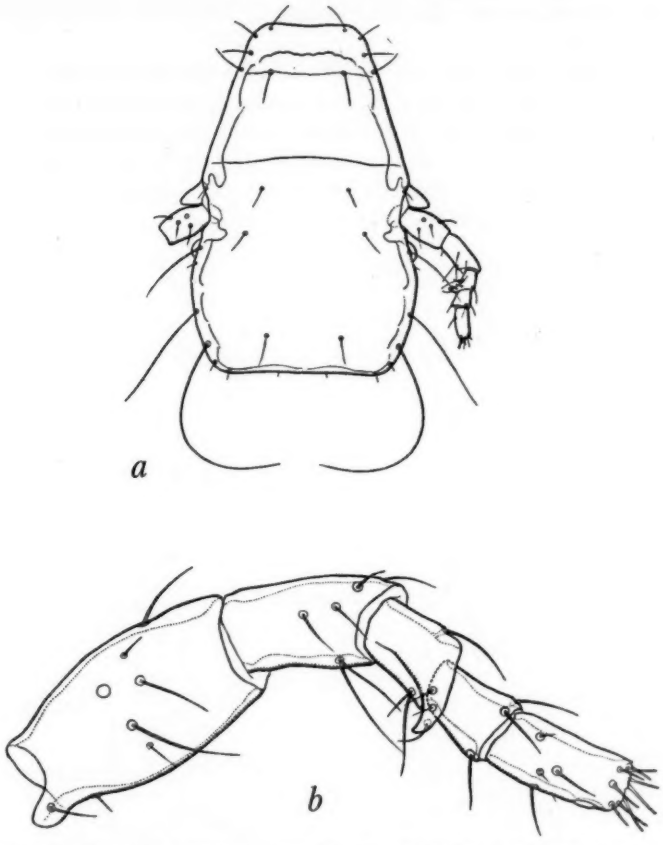


Fig. 2. *Rhynonirmus scolopacis* (Denny). *a*, head of male; *b*, antenna of male.

**Rhynonirmus helvolus** (Nitzsch) 1838

*Lipeurus helvolus* Nitzsch, in Burmeister, *Handbuch*, 1838, **2**, p. 433.
*Lipeurus helvolus* Giebel, *Ins. Epiz.* 1874, p. 229. Pl. xvi, figs. 10–11.
*Lipeurus helvolus* Piaget, *Les Pédiculines*, 1880, p. 375.
*Esthiopterum helvolus* Harrison, *Parasitology*, 1916, **9**, p. 136.

*Type host.* *Scolopax rusticola rusticola* Linn.

*Specimens examined.* 1 ♀ and 2 ♂♂ off *S. r. rusticola* Linn., Macedonia, Vetrina, 23. xi. 1918 (J. Waterston Coll.).

*Description*

Length: ♀ 1·98 mm.; ♂ 1·7–1·8 mm. Breadth (5th seg.): ♀ 0·53 mm.; ♂ 0·46–0·48 mm.

♀. *Head* (see Fig. 3*a*). Forehead distinctly (about one-third) longer than

hindhead. Antenna as in *infuscatus* (Osborn). Trabecula a little more than half the first joint.

*Thorax.* Prothorax over twice as broad as long, mesometathorax about twice as broad as long. Bristles long of prothorax reaching back across the metathorax, all of which ten (5, 5) bristles, except the outermost one on each side, are strong and reach back to the hind edge of the fifth segment of the abdomen.

*Abdomen.* One-third longer than rest of body.

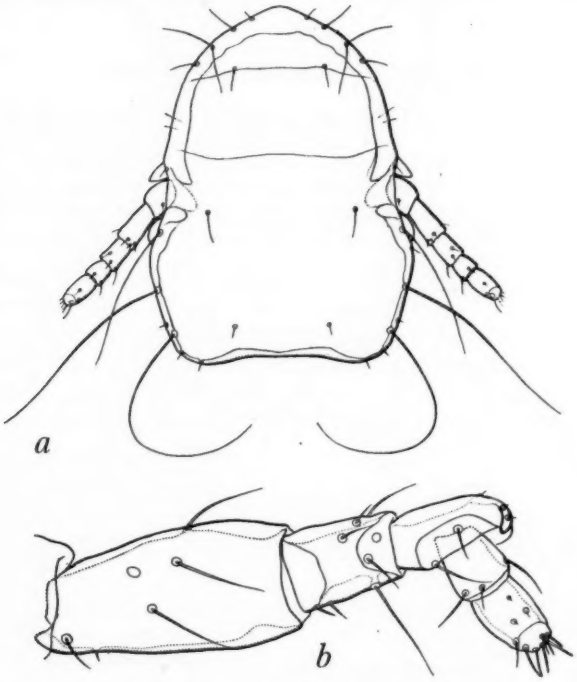


Fig. 3. *Rhynonirmus helvolus* (Nitzsch). *a*, head of female; *b*, antenna of male.

♂. *Head.* Antenna (see Fig. 3*b*). Trabecula about two-sevenths the first joint.
*Thorax.* Metathoracic bristles up to 7, 7 of which four are generally major.
*Abdomen.* In proportion to rest of body 17 : 5.

*Chaetotaxy*

*Abdomen. Upper surface. In both sexes* the posterior row on tergites 2–8 contains only two bristles (1, 1), but the bristles at the sides are as in *infuscatus* (Osborn).

♀. Tergites 8–9 like *infuscatus* (Osborn), but with an extra pair of long bristles laterally behind one half.

♂. The very long and strong bristle between tergites 9 and 10, lateral in

*infuscatus* (Osborn), is inwardly displaced with its two small accompanying bristles.

*Under surface.* Sternites 1 and 2 (and in the male sometimes sternite 3) have only two bristles, sternites 3–5 have four bristles (2, 2), otherwise like *infuscatus* (Osborn).

♂. *Genitalia* (see Fig. 4*b*). Paramere concave on inner edge in its proximal half and slightly exceeding the mesosome in length.

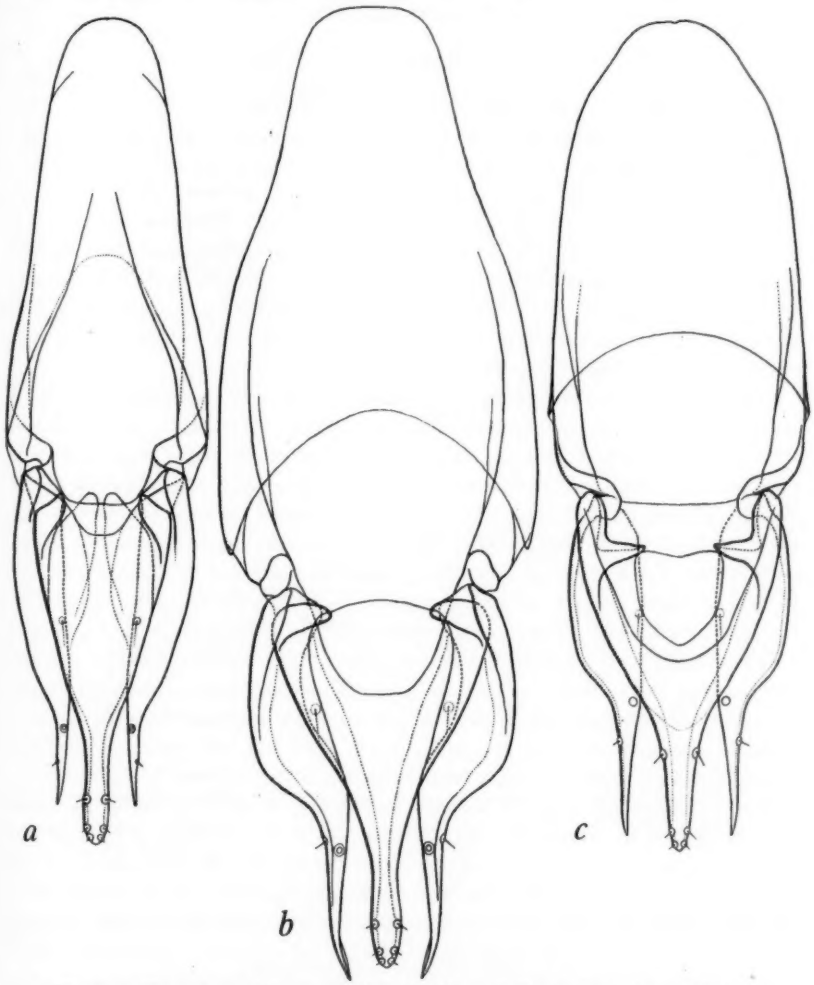


Fig. 4. *a*, *Rhynonirmus infuscatus* (Osborn), male genitalia; *b*, *Rhynonirmus helvolus* (Nitzsch), male genitalia; *c*, *Rhynonirmus scolopacis* (Denny), male genitalia.

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# THE STRUCTURE AND FUNCTION OF THE SPIRACLES OF THE TICK, *ORNITHODOROS MOUBATA* MURRAY

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(With 2 Figures in the Text)

## (*a*) Structure of the spiracle

Cunliffe (1921) has shown that the various stages of *Ornithodoros moubata* may be distinguished by slight differences in the shape of the spiracles. Nuttall, Cooper and Robinson (1908) have described in detail the structure of the spiracles of another tick, *Haemaphysalis punctata*. Otherwise the spiracles of ticks have received little attention except from systematists, and though Nuttall and his collaborators described a closing mechanism in *Haemaphysalis*, no study of its function was made. In this paper I propose to describe the structure of the spiracle of *Ornithodoros moubata*, and show how its function may be investigated by experiments.

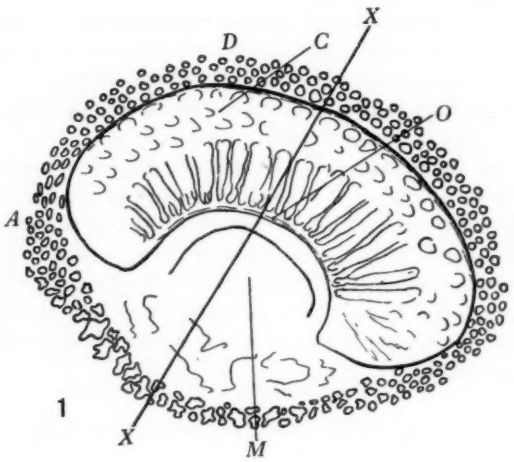
*O. moubata* possesses one pair of spiracles, situated laterally above the third pair of legs. Their external appearance is similar to Fig. 1 in the adults and in all but the first-stage nymphs. There is a semicircular cribriform plate (*C*), inserted into which is the macula (*M*), which is a smooth area of thickened cuticle. The ostium (*O*), a slit-like opening, is situated between the macula and the cribriform plate. Fig. 2 shows the spiracle in section, cut vertically to the surface (see *XX* in Fig. 1). The cribriform plate has three layers. There is the thickened base, a layer of rod-like pedicels (*P*)—some of these appear by transparency in Fig. 1, and a thin external layer supported by the pedicels. The external layer is pierced by minute pores, which connect directly with the atrium (*At*), into which the tracheae open. The direct connection between the pores in the cribriform plate and the atrium is not apparent in Fig. 2, but it must be remembered that the pedicels are rods and not septa, so that the spaces between them are continuous with the atrium. There are muscles attached to the cuticle near the macula, contraction of which presumably closes the ostium, as in *Haemaphysalis*. Relaxation of the muscles will allow the ostium to open, owing to the elasticity of the body wall. The shape of the spiracle is quite different from that of *Haemaphysalis*, but its structure is fundamentally the same, and it seems likely that it works in a similar manner.

## (*b*) Function of the spiracle

An apparatus has been described elsewhere (Mellanby, 1934) in which quantities of water of the order of one-hundredth of a milligram can be weighed, when they are evaporated from an insect. With the same apparatus,

insects can be exposed to various mixtures of gases, and the effects on their water-loss shown.

A batch of fifty second-stage nymphs of *Ornithodoros*, which had been fed 10 days before and then kept at 25° C., was placed in the apparatus mentioned above. They weighed 91 mg. at the start of the experiment, and they lost water into dry air at 17° C. at the rate of two-hundredths of a milligram per hour.



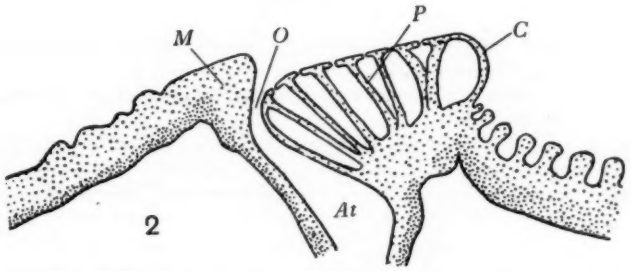




Fig. 1. Surface view of spiracle of *Ornithodoros moubata*. *A*, anterior; *C*, cribriform plate; *D*, dorsal; *M*, macula; *O*, ostium; *XX*, section shown in Fig. 2 cut in this plane.

Fig. 2. Section through spiracle. Cuticular structures only shown. *At*, atrium; *P*, pedicel. Other letters as in Fig. 1.

When they were exposed to dry air containing 5 per cent. of carbon dioxide, they lost approximately seven-hundredths of a milligram of water per hour. The experiment was repeated several times using the same batch of nymphs, and also using other batches, and substantially the same result was obtained every time: in each experiment the *Ornithodoros* lost water much more rapidly into dry air containing 5 per cent. of carbon dioxide than into ordinary dry air.

When insects which possess a mechanism for closing their spiracles are exposed to air containing 5 per cent. of carbon dioxide, they keep their spiracles permanently open (Hazelhoff, 1927) and lose water rapidly by evaporation from their tracheal systems (Mellanby, 1934). The cuticles of many insects appear to be relatively impervious to water vapour. The experiments described above indicate that *Ornithodoros* behaves in the same way as the insects, and so the following conclusions may be drawn:

(1) *Ornithodoros* possesses a mechanism which appears from a morphological examination to be suitable for closing the ostia of its spiracles, and experiments indicate that the spiracles are actually opened and closed.

(2) The physiological reaction which governs the opening and closing of the spiracles is similar to that found in insects.

(3) At room temperature, *Ornithodoros* keeps its spiracles closed for a substantial part of the time. It probably breathes during the remainder of the time by diffusion through the cribriform plate (as suggested by Nuttall, Cooper and Robinson, 1908), but this has not been proved.

(4) The increase in the rate of loss of water which occurs when the spiracles are permanently open implies that water which is lost by evaporation from *Ornithodoros* is lost principally from the tracheal system, and the cuticle is relatively impervious.

I am indebted to H. N. Mellanby for preparing sections of *Ornithodoros*.

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# STUDIES ON THE INFLUENCE OF THE ENVIRONMENT ON THE SHEEP BLOW-FLY, *LUCILIA SERICATA* MEIG.

## II. THE INFLUENCE OF HUMIDITY AND TEMPERATURE ON PREPUPAE AND PUPAE

By A. C. EVANS

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(With 6 Figures in the Text)

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### I. Introduction

In the first paper of this series (Evans, 1934) the effect of temperature and humidity on the egg of *Lucilia sericata* was discussed. In the present paper the effect of the same two climatic factors on the prepupal and pupal stages is studied. While it may seem illogical to follow a study of the egg with a study of the prepupa and pupa, it has been thought advisable to do so, since these three stages are affected directly by the climate of their surroundings; all three stages are non-feeding and so complications arising from the effect of climate on the food, *i.e.* desiccation of carrion, do not arise.

Owing to the writer taking up another position, it has been found impossible to do any work at all on the larvae of this species, and he is conscious of omissions in this and the succeeding papers caused by limitations of time. He is greatly indebted to the Agricultural Research Council for a grant which defrayed the cost of these investigations.

### II. Technique

The prepupae used for experiments were confined in small round boxes (2 in. in diameter by 1 in. depth), the floors and walls of which were made of fine bronze gauze. Each box was filled with very fine pebbles and closed with muslin secured by a rubber band. The junction of the muslin with the edges of the gauze was sealed with wax to prevent any escape from the box, since

the prepupa is very mobile and active. The pupae were kept in cylinders (1·5 in. in diameter by 1 in. depth) of perforated zinc with tops and bottoms of mosquito netting. Control of temperature was secured by means of incubators and constant-temperature rooms in which were kept desiccators containing mixtures of sulphuric acid and water giving the required humidities.

### III. Effect of humidity on:

#### (*a*) *Wet and dry weights*

The effect of humidity on the wet weight of prepupae and pupae is similar to that exerted on the egg; at low humidities weight is lost more rapidly than

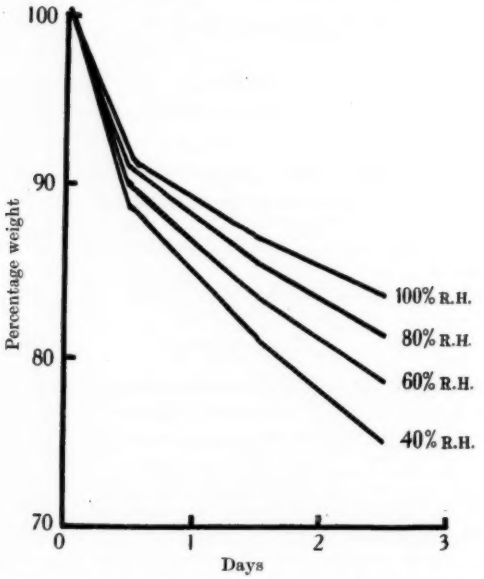


Fig. 1. Progressive loss of weight of prepupae at different humidities at 23° C.

at high (Figs. 1 and 2). At 22° C. the prepupae lose weight rapidly during the first 12 hours and then more slowly until pupation occurs. The pupae also lose weight more rapidly at the beginning of the pupal period, then more slowly, then again more rapidly (Fig. 2). Figures published by Cousin (1932) suggest that the period of rapid loss of weight at the commencement of the pupal stage is continuous with the final period of slower rate of loss of weight in the prepupal stage. The increased loss of weight at lower humidities is clearly due to an increased loss of water, since, as will be shown below, the amount of dry matter consumed during each stage is the same at all humidities.

The increased loss of water at low humidities, coupled with a loss of dry matter which is the same at all humidities, leads, in both stages, to an increase

in the percentage dry weight of the individuals subjected to the conditions; the lower the humidity the higher the proportion of dry matter becomes (Table I).

The relation between humidity and loss of water and increase in percentage dry weight has been studied in detail with pupae, this stage lending itself to greater accuracy than the prepupal stage because of its immobility and ease of handling. Fig. 3 shows the relation between the percentage loss of water

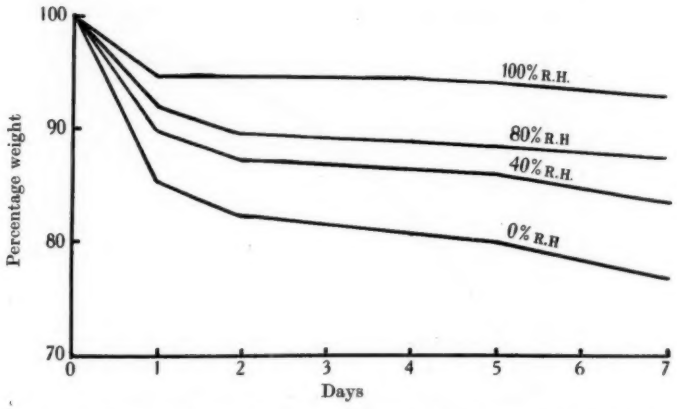


Fig. 2. Progressive loss of weight of pupae at different humidities at 23° C.

Table I. *Showing the change in percentage dry weight due to humidity acting throughout the whole of the prepupal and pupal stages at* 23° C.

| | | Percentage dry weight | | | | |
|---|---|---|---|---|---|---|
| | | Final Relative humidity | | | | |
| Stage | Initial | 100 | 80 | 60 | 40 | 0 |
| Prepupae | 30·9 | 31·8 | 32·4 | 33·2 | 36·2 | — |
| Pupae | 29·6 | 29·6 | 30·5 | — | 32·9 | 34·0 |

and humidity; each point is the mean of five readings on batches of fifteen pupae of the same age and from the same culture. It was suggested in the case of the egg of *Lucilia* (Evans, 1934) that the relation between percentage loss of water and humidity is linear; in the case of the pupae it is clearly parabolic, an excellent fit to the experimental data being given by the formula

$$y = 1{\cdot}26x - 0{\cdot}34x^2 - 0{\cdot}1,$$

when $y$ = percentage loss of water and $x$ = humidity expressed as saturation deficiency (Table II).

### (*b*) *Duration of prepupal and pupal stages*

The effect of temperature on the duration of the prepupal and pupal stages has been studied by Wardle (1930) and Cousin (1932) and needs no further comment. The effect of humidity on these stages has not been so carefully studied. Wardle (1930) gives figures for the duration of the prepupal stage at 30° C. and five different soil moisture contents, but the duration values are so scattered that no conclusion can be drawn. The same author states that humidity has no appreciable effect on the length of the pupal period.

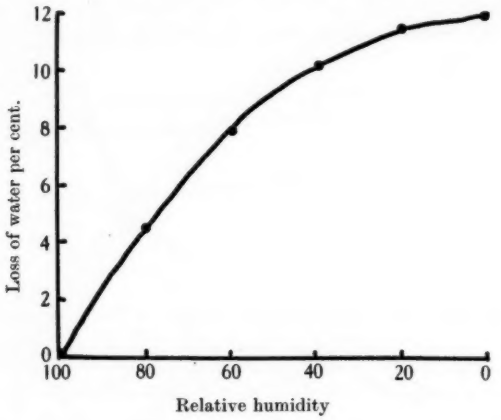


Fig. 3. The relation between percentage loss of water by pupae and humidity at 23° C.

Table II. *Relation between percentage loss of water of pupae and humidity expressed in mm.* (Hg) *saturation deficiency at* 23° C. *over a period of* 4 *days.*

| Saturation deficiency | Percentage loss of water | |
|---|---|---|
| | Found | Calculated |
| 0 | 0 | −0·1 |
| 4 | 4·4 | 4·4 |
| 8 | 7·8 | 7·8 |
| 12 | 10·2 | 10·2 |
| 16 | 11·4 | 11·4 |
| 20 | 11·7 | 11·7 |

Humidity has but little effect on the duration of the prepupal and pupal stages compared with its effect on the rate of development of the egg. The prepupae seem to pupate more rapidly at low than at high humidities (Table III). This result was obtained in all the experiments performed.

The same relation has been found for *Phormia* sp. R.-D. at 33° C. A relative humidity of 100 per cent. greatly retards the rate of pupation of *Calliphora erythrocephala* Meig. and *Sarcophaga falculata* Pand.

The pupae develop most rapidly at median humidities, showing a distinct retardation at high and low humidities (Fig. 4).

### (c) *Consumption of dry matter*

The slight effect of humidity on the duration values of the prepupal and pupal stages would hardly be expected to involve an appreciable increase in

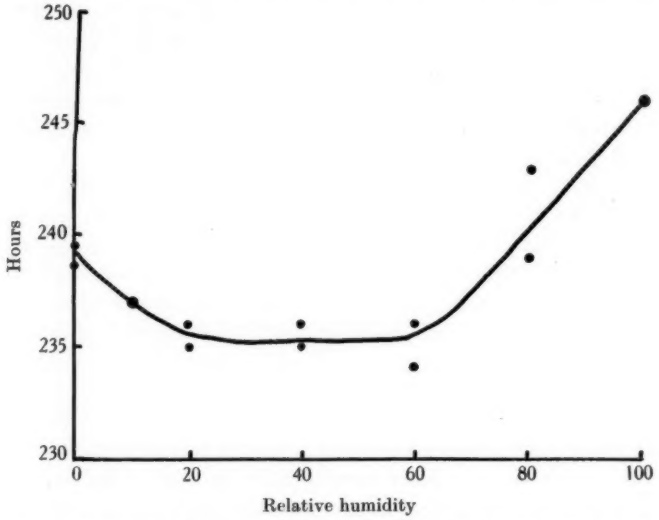


Fig. 4. The relation between humidity and duration of the pupal period at 23° C. Points indicate means of groups of fifty individuals.

Table III. *Relation between humidity and rate of pupation at* 25° C. *expressed as percentage of prepupae pupated after definite intervals of time.*

| Age of prepupae in hours | Relative humidity (%) | | | |
|---|---|---|---|---|
| | 100 | 80 | 40 | 0 |
| 70 | 32 | 36 | 36 | 74 |
| 75 | 70 | 82 | , | 92 |
| 80 | 78 | 96 | 96 | 98 |

Table IV. *Grams of dry matter (progressive totals) consumed by* 100 *g. of fresh material during prepupal and pupal stages, at* 23° C.

| Stage | Day | Relative humidity (%) | | | | |
|---|---|---|---|---|---|---|
| | | 100 | 80 | 60 | 40 | 0 |
| Prepupae | ½ | 2·0 | 1·8 | 3·0 | 2·4 | — |
| | 1½ | 3·7 | 3·9 | 3·5 | 3·5 | — |
| | 2½ | 4·5 | 4·7 | 4·9 | 3·9 | — |
| Pupae | 2 | — | 1·2 | — | 1·4 | 1·2 |
| | 4 | — | 1·7 | — | 1·7 | 1·4 |
| | 6 | — | 2·5 | — | 2·2 | 2·7 |
| | 8 | — | 3·4 | — | 3·4 | 4·0 |

the amount of dry matter consumed under different conditions of humidity. Table IV shows that this assumption is correct.

### IV. Effect of temperature and humidity upon survival

Wardle (1930) makes brief comments on the effect of temperature and soil moisture content on the survival of prepupae and pupae. At twenty-two out of thirty-five combinations of temperature and soil moisture content, more than 70 per cent. of the prepupae died and in no case did 100 per cent. survive. The occurrence of a percentage mortality in all batches is attributed to some internal factor of the organism such as accumulation of uric acid in the tissues. The same investigator also found that humidity had no appreciable effect on the survival in the pupal stage.

The results of the present investigation do not support the findings of Wardle. Figs. 5 and 6 give the percentage survival of prepupae and pupae at numerous combinations of temperature and humidity, embracing all humidities over the temperature range of this species. Duplicate batches of twenty-five individuals were found to give very comparable results so long as they were obtained from one source. In a preliminary experiment larger numbers were used, but no better results were obtained than with twenty-five individuals per batch. Humidity exerts no influence upon the survival of prepupae between 20 and 30° C. Outside these limits, humidity has a definite effect; high temperatures and high saturation deficiencies bring about death rapidly through desiccation. Low temperatures and low saturation deficiencies bring about death slowly through desiccation, since the prepupae are subject to the dry atmosphere for long periods of time. In a saturated atmosphere at low temperatures, the prepupae enter a wet diapause which can only be broken by removing them to dry conditions; if they are kept constantly in saturated air they die after several months. It is interesting to note that a prepupal diapause was not induced in these experiments by completely dry air as recorded by Cousin (1932).

The effect of humidity on the pupae is shown in Fig. 6. It will be seen that the unfavourable effect of drier air is greater than on the prepupae. At the higher saturation deficiencies at all temperatures, the effect of humidity is to bring about a certain mortality depending upon the temperature. This mortality of the pupae is brought about in two ways: failure to develop and failure to emerge. Non-development is brought about by a greater loss of water than the organism can withstand and death usually occurs early in the pupal stage; failure to emerge would seem to be caused by the too rapid drying up of the moulting fluid secreted just before emergence occurs. As in the case of the eggs of this species, so with the pupae, development occurs over a wider range of temperature and humidity than does emergence. The broken line in Fig. 6 encloses the area over which 100 per cent. survival takes place, and the continuous line the area over which 100 per cent. development takes

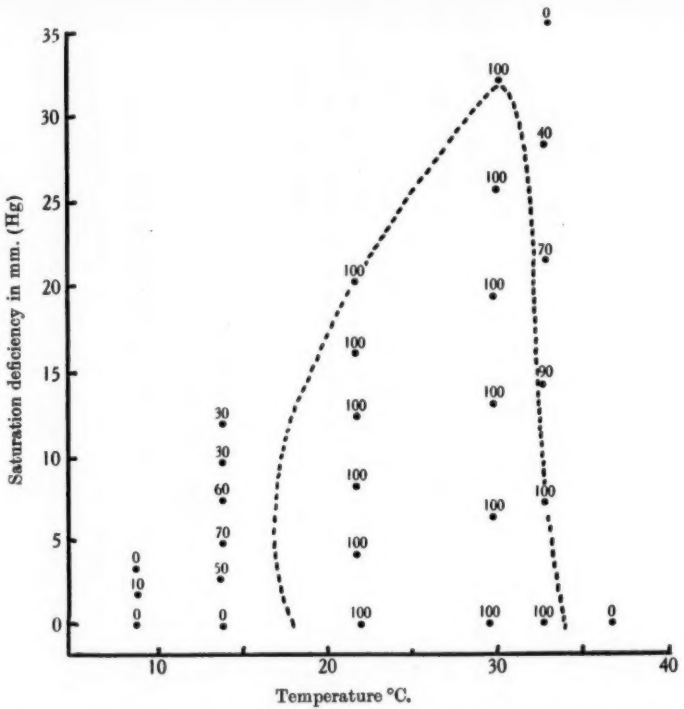


Fig. 5. Mortality limits of the prepupae at different combinations of temperature and humidity. Figures represent the percentage pupation.

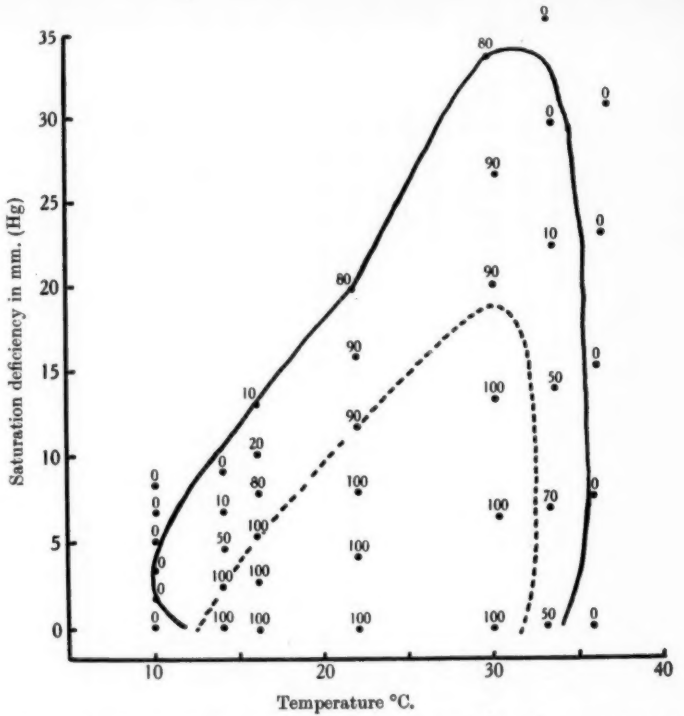


Fig. 6. Mortality limits of the pupae at different combinations of temperature and humidity. Figures represent the percentage emergence. The discontinuous line encloses the region of 100 per cent. emergence, the continuous line that of 100 per cent. development.

place. Evidently the mortality which occurs in the pupal stage is chiefly brought about by the effect of humidity upon the mechanism of emergence, since numbers of flies die after breaking the puparial cap with their ptilinum.

It is customary when working on the metamorphic stage of the higher Diptera to deal separately with the prepupal and pupal stages. Although this procedure has been justified by the results obtained, the fact that these two stages are parts of one process must not be lost sight of. An experiment has therefore been carried out dealing with the effect of humidity at the critical temperature of 33° C. upon the two stages running concurrently. At this temperature the continued effect of humidity on pupae derived from prepupae kept at the same humidity is striking (Table V).

Table V. *Effect of humidity upon the survival* (*a*) *and development* (*b*) *of pupae alone, and prepupae and pupae maintained continuously at* 33° C.

| | Relative humidity (%) | | | | | |
|---|---|---|---|---|---|---|
| | 100 | 80 | 60 | 40 | 20 | 0 |
| | (*a*) Survival (%) | | | | | |
| Pupae alone subjected | 52 | 72 | 50 | 14 | 0 | 0 |
| Prepupae and pupae subjected | 0 | 4 | 0 | 2 | 2 | 0 |
| | (*b*) Development (%) | | | | | |
| Pupae alone subjected | 92 | 100 | 98 | 98 | — | 94 |
| Prepupae and pupae subjected | 48 | 62 | 36 | 34 | 22 | 0 |

Thus a much higher mortality is found considering the metamorphic period as a whole. This experiment shows also that no acclimatisation to humidity occurs in the prepupal stage, enabling the pupae to withstand lower humidities than they might otherwise have done. It would be interesting to extend this study so as to include lower temperatures.

## V. Summary

Prepupae and pupae are shown to lose weight more rapidly at low than at high humidities. This more rapid loss of weight is due to loss of water alone and not to loss of dry matter.

The limits of temperature and humidity which each of the two stages can survive have been mapped out. If the metamorphic period as a whole is considered, it is likely that the limits of temperature and humidity for survival and development will be narrower than for each stage considered alone.

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# STUDIES ON THE INFLUENCE OF THE ENVIRONMENT ON THE SHEEP BLOW-FLY, *LUCILIA SERICATA* MEIG.

## III. THE INFLUENCE OF HUMIDITY AND TEMPERATURE ON THE ADULT

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(With 4 Figures in the Text)

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### I. Introduction

The effect of climate on the adult blow-fly is much more complex than on the immature stages because of the manifold activities of the adults. The ecological niches occupied by the immature stages are more strictly limited than those of the adult, whose powers of flight, increased sensitivity, choice of food, etc., permit to it a greater freedom. A knowledge of the effect of climate on the adult is especially desirable if directed towards its influence on the rate of reproduction of the fly both directly and indirectly through its effect on competing species. This latter effect is especially important and deserving of more attention than it has been possible to give during these investigations.

The methods used in this investigation are those described by Buxton and Mellanby (1934).

I am greatly indebted to the Agricultural Research Council for a grant defraying the cost of these investigations.

### II. The effect of humidity on:

#### (*a*) *The survival of flies fed on different diets for varying periods of time*

Graham-Smith (1916) and Wardle (1927) have studied the effects of climate on populations of wild flies. Both record a high mortality amongst recently emerged flies in nature, this mortality not being found in flies bred and reared

in the laboratory. It was thought that this mortality might be caused by differences in the state of nutrition of the flies, as young flies do not contain so much reserve material as older ones (Evans, 1935), and therefore might be expected to succumb more easily to adverse conditions. To test this hypothesis the length of life of unfed flies and of flies fed on various diets for 1 day and for 6 days (when eggs are ready to be laid) was recorded at several humidities. The results described below do not support the hypothesis, as unfed flies live nearly as long as flies fed for 1 day on certain diets and much longer than most flies fed for 6 days on the same diets.

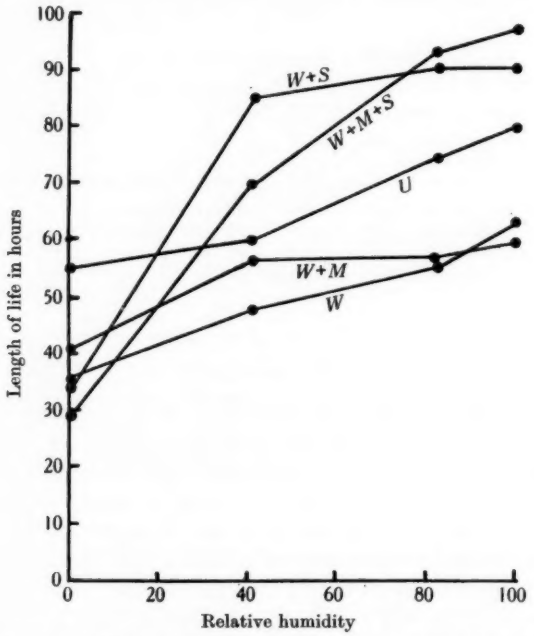


Fig. 1. Length of life of unfed flies and flies fed for 24 hours on various diets, 23° C. $W$ = water, $M$ = meat, $S$ = sugar, $U$ = unfed. All flies 24 hours old at commencement of experiment.

Unfed flies (Fig. 1, $U$) and flies fed on growing diets (meat, sugar and water; sugar and water, Fig. 1, $W+M+S$ and $W+S$) and on non-growing diets (meat and water; water alone, Fig. 1, $W+M$ and $W$) die more quickly at low than at high humidities. Life is longest at relative humidities of 100 and 80 per cent., the flies evidently dying of starvation. On the whole, flies fed on growing diets live longest, while unfed flies live longer than flies fed on non-growing diets. This can be understood in view of the facts given by Evans (1935); flies fed on growing diets rapidly lay down extra reserve material while flies fed on non-growing diets lose some of the reserve material present in the newly emerged fly. Again, the rate of metabolism of unfed flies is

distinctly lower than that of fed flies. This fact also accounts for the unfed flies living longer than those fed on non-growing diets. No difference was observed between the length of life of the sexes. Buxton and Lewis (1934) found that for *Glossina tachinoides* and *G. submorsitans*, life is longer at intermediate humidities than in very dry or moist air both for unfed and fed flies.

The reaction to humidity of flies fed for 6 days differs distinctly from that of flies fed for 1 day (Fig. 2). By this time unfed flies and flies fed on non-growing diets are dead. In flies fed for 6 days on growing diets, the maximum length of life occurs at 80 per cent. relative humidity, saturated or nearly

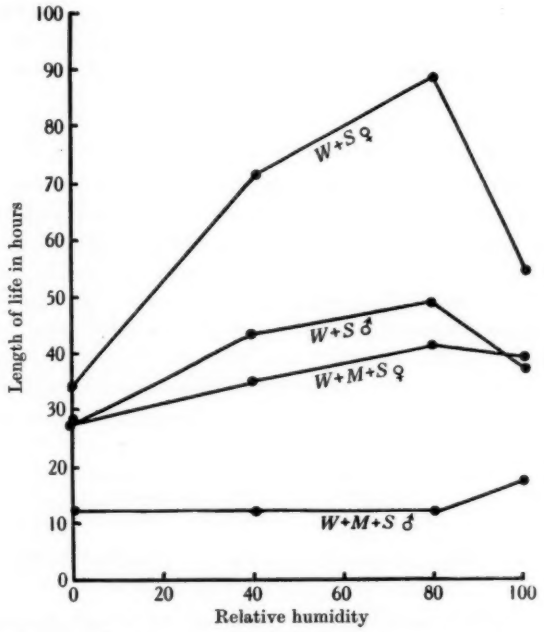


Fig. 2. Length of life of flies fed on growing diets for 6 days, 23° C. $W$ = water, $M$ = meat, $S$ = sugar. All flies 6 days old at commencement of experiment.

saturated air being distinctly injurious. By surrounding the cage in which the flies are kept with damp blotting paper, the length of life is less than half that recorded if the cage is kept in a jar containing distilled water. The length of life at 100 per cent. relative humidity, given in Fig. 2, was recorded under the latter conditions. This susceptibility to saturated air is the first interesting difference between flies fed for 6 days and those fed for 1 day. The second difference is that, in flies fed for 1 day on growing diets, diet has but little influence on the length of life of the flies at each humidity (Fig. 1, $W+M+S$ and $W+S$), whereas in flies fed for 6 days on growing diets, the diet has a

distinct influence on the length of life. Flies fed on sugar and water live longer than those fed on meat, sugar and water. Thirdly, a striking sex difference is apparent, the females living much longer than the males fed on the same diet. Comparing Figs. 1 and 2, the length of life at all humidities of females fed for 6 days on sugar and water is found to be about equal to that of both sexes fed on meat, sugar and water, and on sugar and water, for 1 day; but the length of life of males fed on sugar and water, and of males and females fed on meat, sugar and water for 6 days is distinctly less than that of flies fed on the same diets for 1 day.

The differences in length of life at constant humidity of the flies fed for 6 days on growing diets are correlated with the percentage amount of reserve material present. Females and males fed on sugar and water contain respectively 38 and 33·8 per cent. of dry matter, and when fed on meat, sugar and water 33·0 and 31·2 per cent.

### (*b*) *The wet and dry weights of flies fed on growing diets*

Although there is a direct correlation between the percentage dry weight of flies fed on growing diets for 6 days and their length of life at one humidity, it is not to be expected that this correlation will provide a complete explanation, since the reserves laid down by flies fed on sugar and water differ from those laid down by flies fed on meat, sugar and water. An analysis of the fat and carbohydrate content of flies fed on these diets is given in Table I.

Table I. *Percentage fat and carbohydrate contents of flies.*

| | | Wet weight | | Dry weight | |
|---|---|---|---|---|---|
| Fed | Sex | Fat | Carbohydrate as glucose | Fat | Carbohydrate as glucose |
| Sugar and water | Female | 1·1 | 14·8 | 3·3 | 40·9 |
| | Male | 1·5 | 7·3 | 5·0 | 24·5 |
| Meat, sugar and water | Female | 2·0 | 7·5 | 6·6 | 24·6 |
| | Male | 1·7 | 4·4 | 5·9 | 15·5 |

Fat was estimated as ether-extractible matter and carbohydrate by the Issekutz and Both (1927) modification of the Hagedorn-Jensen method. The fat content of flies given meat as part of their diet is slightly higher than that of flies fed on sugar and water, but the differences are hardly significant. However, the carbohydrate content of flies fed on sugar and water is higher than that of flies fed on meat, sugar and water. The females contain considerably more carbohydrate than the males, thus giving a direct correlation between length of life and carbohydrate content. The value of carbohydrate in prolonging the length of life of fully fed flies under adverse conditions (40 per cent. relative humidity at 23° C.) can be demonstrated by allowing the flies to feed on a lump of sugar kept in the cage. Flies feeding on sugar live 2–3 times as long as controls without sugar. Unfortunately no figures for the protein content of flies fed on the two growing diets are available, but there

can be no doubt that the protein content of flies fed on meat, sugar and water is considerably higher than that of flies fed on sugar and water.

Another contributory cause for the shorter life of flies fed on meat, sugar and water is that they lose weight more rapidly at all humidities than flies fed on sugar and water. Figs. 3 and 4 give the loss of weight over a period of 12 hours of fully fed females; the loss of weight is analysed into two curves—loss of water and loss of dry matter. The rate of loss of water and of dry matter is greater at low than at high humidities in flies fed on both diets.

During this short period of 12 hours, certain changes in the percentage dry weight occur. In flies fed on sugar and water the percentage dry weight decreases appreciably from 36·3 to 31·1 and 33·7 per cent. at 100 and 80 per cent. relative humidity respectively, while at 0 per cent. relative humidity it remains constant at 36·4 per cent. In flies fed on meat, sugar and water, the percentage dry weight decreases from 37·0 to 33·0 per cent. at 100 per cent. relative humidity and increases slightly to 38·0 and 37·7 per cent. at 50 and 0 per cent. relative humidity. Over longer periods of time (1–5 days), the tendency of flies fed on sugar and water to decrease their dry weight and of flies fed on meat, sugar and water to increase their dry weight becomes accentuated; at 40 per cent. relative humidity females fed on sugar and water decrease their percentage dry weight by 8·0 per cent. and females fed on meat, sugar and water increase their percentage dry weight by 4·3 per cent. In these changes in dry weight on exposure to different humidities, *Lucilia* differs markedly from *Glossina*. Buxton and Lewis (1934) record that although unfed tsetse flies lost more weight at low than at high humidities, the final percentage dry weight was much the same as the initial percentage dry weight. They attribute this regulation to a fat-combustion mechanism. The reserve material of this fly is largely fat, this substance accounting for 25–30 per cent. of its dry weight as against 5–7 per cent. in *Lucilia*.

### (c) *The length of life of flies emerging from pupae kept at various conditions of humidity*

It might be thought that the climatic conditions prevailing during the pupal period would have some effect on the reaction to humidity of the resulting adults, enabling them either to withstand adverse conditions through acclimation or causing them to succumb more readily through more rapid desiccation. The experiments discussed below, however, discount this idea completely. Unfed flies emerged from pupae kept at either 0 or 95 per cent. relative humidity live for equal periods of time both at 0 and 50 per cent. relative humidity (Table II).

Table II. *Effect of humidity on length of life of unfed adults emerged from pupae kept at* 0 *and* 95 *per cent. relative humidity at* 23° C.

| Adults kept at (% R.H.) | 0 | | 50 | |
|---|---|---|---|---|
| Pupae kept at (% R.H.) | 0 | 95 | 0 | 95 |
| Mean length of life (hours) | 48 | 42 | 54 | 58 |

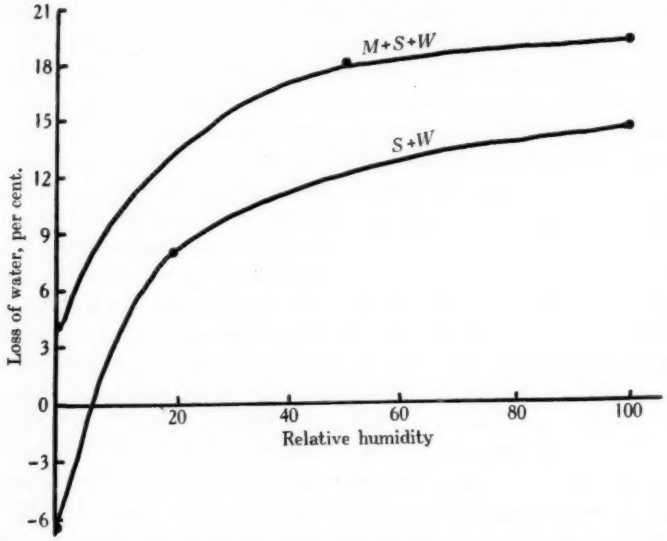


Fig. 3. Relation between loss of water and humidity in flies fed on a diet of meat, sugar and water, and flies fed on sugar and water, 23° C.

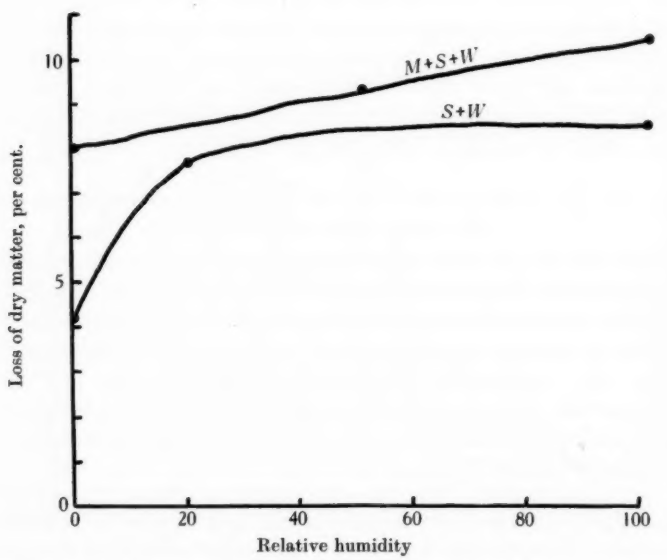


Fig. 4. Relation between loss of dry matter and humidity in flies fed on a diet of meat, sugar and water, and flies fed on sugar and water, 23° C.

In the case of fed flies, the great increase in percentage dry weight which occurs on feeding completely nullifies the differences in dry weight of the freshly emerged flies caused by the action of humidity upon the pupae (Table III).

Table III. *Effect of feeding for 3 days on a complete diet on the water balance of the adult flies emerged from pupae kept at various humidities.*

| Pupae kept at (% R.H.) | Percentage dry weight of | |
|---|---|---|
| | Unfed flies | Fed flies |
| 100 | 25·9 | 38·0 |
| 80 | 26·3 | 33·1 |
| 40 | 28·9 | 35·9 |
| 0 | 31·4 | 34·9 |

### (*d*) *The development of the ovaries and oviposition*

In addition to studies of the effect of climate on the organism as a whole, data dealing with its effect on the various physiological processes within the individual are urgently needed. Several authors (Headlee, 1917; Adolf, 1920; Schubert, 1928) have dealt with the effect of humidity on the number of eggs laid by various insects without distinguishing between the effect of humidity on the maturation of the ovaries and on oviposition. The data presented below are somewhat fragmentary in nature but show that in the case of *Lucilia* it is very necessary to distinguish between the effect of humidity on the maturation of the gonads and on oviposition.

The females used in these experiments were kept in batches of twenty in celluloid cages at relative humidities of 100, 80, 40 and 0 per cent. at 23° C. They were fed on various diets. The problem of feeding flies kept in closed jars without interfering too much with the control of humidity was found to be difficult. In the first instance, unfed flies 1 day old were allowed to feed for 4 hours on fresh meat and were then transferred to the cages, each of which contained a lump of sugar. This method of providing adequate carbohydrate has been found quite satisfactory. In order to provide the nitrogenous substances necessary for growth of the ovaries, the cages were removed from the jars and the flies allowed to feed on fresh meat for 1 hour per day. Meat could not be kept in the jars continuously, as this would interfere seriously with the control of humidity. This method proved unsatisfactory on account of the high mortality at all humidities. On the seventh day, when the flies were dissected for examination of the ovaries, twelve, nine and seven flies were alive at 100, 80 and 40 per cent. relative humidity respectively. At 0 per cent. relative humidity, all the flies had died by the end of the third day. Examination of the stage of growth attained by the ovaries by the seventh day showed that high humidities favoured growth while low humidities were unfavourable. The following is a rough approximation of the development of the ovaries on the seventh day.

Controls fed on meat continuously, ovaries fully developed in twenty flies.
At R.H. 100 per cent., ovaries half to fully developed in twelve flies.
„ 80 „ „ half developed in nine flies.
„ 40 „ „ quarter to half developed in seven flies.

Further experiments were carried out on methods of supplying a more concentrated supply of nitrogenous substances and to cut down the high mortality. Liver was substituted for meat as the daily feed but proved unsatisfactory. The method finally adopted was to mix the nitrogenous substances with sugar dissolved by heat in its own water of crystallisation. Dried nitrogenous substances such as blood, blood albumen and casein proved unsatisfactory, but liver mixed in this way with its own weight of sugar and then dried down proved excellent. It could be placed in small amounts in the cages and kept there continuously, as it did not deliquesce and so bring about death of the flies by "drowning" in a sticky mess.

The technique finally adopted was to keep a supply of sugar and sugared liver in the cages continuously and to feed the flies daily for an hour on fresh meat. The results obtained by this method support the conclusion stated above, that high humidities are favourable to growth of the ovaries and low humidities are unfavourable. Out of the twenty flies initially present, the following number were alive on the sixth day at 100 and 80 per cent. relative humidity: nineteen and eight respectively, and their ovaries were fully developed. At 40 per cent. relative humidity five were dead by the third day, the remainder died on the fourth day, and their ovaries were one-quarter to one-third developed. All the flies kept at 0 per cent. relative humidity were dead by the second day. This relationship between the growth of the ovaries and humidity would appear to be brought about by differences in the rate of metabolism of flies kept at high and low humidities (Fig. 4), the higher rate of metabolism of flies kept at low humidities lessening the amount of material available for the growth of the ovaries. It is interesting to compare the range of humidity favourable for growth of the ovaries in *Lucilia* with that favourable to reproduction in the tsetse fly. Buxton and Lewis (1934) find that for *Glossina tachinoides* high and low humidities are unfavourable; at 30° C. and 88 per cent. no births at all occur, and at 44 per cent. relative humidity the mean birth-rate is three times that at 19 per cent. relative humidity and nine times that at 11 per cent. relative humidity. For *G. submorsitans* the most favourable relative humidity is 11 per cent.

The effect of humidity upon oviposition is not greatly marked except at high temperatures. The flies were prepared for experimental use as follows. They were allowed to feed on a complete diet until some eggs were laid, the meat was then removed and the flies allowed to feed on sugar and water only for 2 days, when they were used for the experiments. Batches of twenty or twenty-five flies were confined in cages and placed for 3 hours at the conditions of temperature and humidity to be studied. Fresh meat was then introduced and the number of egg batches counted after 2 hours had elapsed

and again when all the flies were dead. Very few batches were laid after 2 hours. The results obtained show that under the above conditions humidity has no effect on oviposition between the temperatures of 10 and 35° C. At 10° C. only one small batch of eggs and a few scattered eggs were laid at each humidity by forty-five flies. Above 35° C. humidity begins to have an effect; at 40° C., forty-five flies laid fifteen, twelve and four batches of eggs at 100, 50 and 0 per cent. relative humidity respectively, all the flies being alive after 5 hours' exposure. At 42° C. no eggs were laid, as the flies were dying rapidly at the end of the preliminary 3 hours' exposure.

## III. SUMMARY

The length of life at various humidities of unfed flies and of flies fed for 1 day and 6 days on diets of meat, sugar and water, sugar and water, meat and water, and water alone, is discussed. The continued feeding of flies on a diet of meat, sugar and water brings about a shortening of their life at all humidities when the flies are starved owing to their increased rate of metabolism. Flies fed on sugar and water are more resistant to humidity than flies fed on meat, sugar and water, and the females are more resistant than the males. Flies fed on meat, sugar and water lose weight more rapidly than flies fed on sugar and water.

High humidity is more favourable for growth of the ovaries than low humidity. Humidity has no effect on oviposition at temperatures of 10–35° C., but at 40° C. low humidity is unfavourable.

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